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SCIENTIFIC MEMOIRS

BY

MEDICAL OFFICERS OF THE ARMY OF INDIA.

EDITED BY

B. SIMPSON, Esq., M.D.,

SURGEON-GENERAL WITH THE GOVERNMENT OF INDIA.

PART II.

1886.

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On the Effects sometimes following injection of Choleraic Comma-bacilli into the Subcutaneous Tissues in Guinea-pigs.

BY

SURGEON-MAJOR D. D. CUNNINGHAM, M.B.,

SPECIAL ASSISTANT TO THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA.

(*With Plate I.*)

During the course of the past few weeks I have met with a series of phenomena in certain experiments on the subcutaneous injection of choleraic comma-bacilli into the bodies of guinea-pigs which appear to be sufficiently remarkable to merit special record. I propose, therefore, in the present paper to give a detailed account of these experiments and their results, along with a few brief notes regarding their apparent significance.

I.—Source of the material employed in the experiments.

The commas employed in all the experiments belonged to a series of cultivations originally derived from a case of cholera which was admitted into the Medical College Hospital on the 21st January 1886. A portion of a fresh evacuation was sent to me for examination on that day. It consisted of a brownish-grey, alkaline, watery fluid, and an abundant sediment of gelatinous, somewhat pinkish flocculi.

The examination of fresh preparations, made immediately on the arrival of the material, showed that it was crowded with schizomycete organisms of various forms. Commas were only present in very small numbers and constituted a very inconspicuous feature as compared with other forms.

In spite of this, however, a plate cultivation yielded what was practically a pure crop of commatous colonies. These were present in innumerable numbers, and the only other form of colonies recognisable were two or three consisting of a very large kind of micrococcus which was present as a conspicuous feature in the original material. This plate afforded the starting-point for a series of successive pure tube cultivations of the commas which were employed in the experiments.

II.—Details of the individual experiments.

Experiment 1.

On the 9th of February a healthy guinea-pig, weighing 482 grammes, received a subcutaneous injection of a little less than 1 c. c. of fluid, con-

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sisting of sterilised 0·8 % salt solution full of commas derived from a pure tube cultivation of commas of four days' standing. The site of the injection was the inner surface of the left thigh. Every precaution was taken to avoid any accidental contamination. The syringe which was employed had been immediately beforehand soaked in strong carbolic acid and then thoroughly washed in rectified spirit and in freshly boiled, distilled water. The site of injection was cleared of hair and carefully washed with solution of corrosive sublimate and with spirit, and the skin was drawn aside ere the introduction of the point of the syringe so as to secure a valvular action on its subsequent release.

On the 10th of February the animal appeared to be unaffected, save that it was slightly lame in the injected extremity. On the 11th of February at 9 A.M., however, it was conspicuously affected. It was lying down, and constantly uttering peculiar sharp cries as though of pain. It evidently felt cold and its respirations were very rapid. There were constantly recurring peculiar twitching movements of the abdominal and aural muscles, and evidences of pain on any pressure of the abdominal walls. In the afternoon at 4 P.M. it was still alive. The general symptoms were much the same as in the morning. It was unable to stand or walk, the eyes were much sunken, the surface of the body sensibly cold to touch, and it was constantly squeaking feebly.

It died during the course of the night, and the body was examined at 10 A.M. on the 12th February. The total body weight was 429 grammes, so that there had been a loss in weight of almost exactly 11 % since the operation. The weights of the body and of various organs are shown in the following table:—

		Of total weight.
Liver	29·0 grammes	=6·7 %
Spleen	1·8 „	=0·419%
Right kidney	3·0 „	=0·69 %
Left „	2·8 „	=0·65 %
Right lung	2·0 „	=0·46 %
Left „	1·9 „	=0·44 %
Heart	3·8 „	=0·88 %

The next table shows the corresponding weights in the body of a healthy guinea-pig in which they were specially determined:—

Total body weight		=452 grammes.
Liver	32·36 grammes	=7·1 %
Spleen	0·44 „	=0·09%
Right kidney	1·86 „	=0·41%
Left „	1·9 „	=0·42%
Right lung	1·64 „	=0·36%
Left „	1·24 „	=0·27%

On comparing the above series of figures it is evident that in this case

there was slight diminution in the relative weight of the liver, and increase in the weights of all the other organs and specially in that of the spleen.

On removing the skin a large patch of sanguineous effusion became visible, thickest over the site of injection and spreading up thence over the lower half of the abdomen on the same side. On opening the abdominal cavity the surface of the peritoneum was found to be coated with a thin stratum of sticky secretion, very similar to that present in many cases of cholera. This caused the surfaces of the viscera to adhere more or less to one another, but there was an absence of fluid, of congestion, or of adhesions of a characteristically peritonitic character. The stomach was pale, and contained a mixture of gelatinous, transparent mucus, and brown grumous matter. The small intestine was empty, the mucous surface being pale and very moist, and here and there covered by patches of brownish mucus. The cæcum contained a considerable quantity of soft, pale umber contents, which seemed to be rather dryer than normal. The reaction was neutral. The rest of the large intestine was pale and empty, save towards the lower end, where one or two formed fæcal pellets were present. The liver was rather pale and the gall-bladder distended with pale-yellowish bile. The spleen was dark red and the kidneys were congested. On opening the thoracic cavity the lungs were found to be pale pink and free from congestion or extravasations. The right side of the heart was distended with blood.

A series of preparations of the materials of the subcutaneous sanguineous effusion, of the peritoneal secretion, the cardiac blood and the contents of the large and small intestine, was made with the following results :—

1.—Subcutaneous sanguineous effusion.
This was full of comma-bacilli.

2. Peritoneal secretion.
This also contained abundant characteristic commas.

3. Cardiac blood.
No commas or other schizomycetes were to be found.

4. Intestinal contents.
The material derived from the small intestine consisted of some of the brownish mucus previously alluded to, obtained from a patch of it situated in the ileum.

It only contained a very few recognisable epithelial elements, the greater part consisting of amorphous mucoid matter. In this basis very large numbers of characteristic small commas were present. Some of them were irregularly scattered through the basis; a large number of them, however, were associated in small groups, and some of these were clearly specially related to large nuclear bodies, the commas being aggregated on and around these (*vide* Plate I, Fig. 3).

In such cases it was clearly evident that one was dealing with bodies parallel to those described by Drs. Klein and Gibbes in connection with the small straight bacilli which they have pointed out as a characteristic feature in choleraic materials. The mucus from the large intestine also contained small commas, but not in at all such large proportion, and the large curved forms normally present there occurred in relatively very small numbers only.

Various cultivations were carried out with the following results:—

1. A plate cultivation of the subcutaneous sanguineous effusion yielded a pure crop of abundant commatous colonies.
2. A plate cultivation of mucus from the ileum yielded a crop of commas mingled with colonies of short, straight bacilli.
3. A plate cultivation of materials from the cæcum gave similar results.
4. A tube cultivation of cardiac blood obtained by direct perforation of the right side of the heart by the point of a freshly-drawn capillary pipette, the point being broken within the cardiac cavity, and direct inoculation of an agar-agar tube being subsequently carried out. This yielded a pure crop of commas.

An extensive series of preparations of various tissues which had been preserved in absolute alcohol was subsequently carefully examined. In spite of varied methods of staining, no traces of the presence of commatous or other schizomycete forms could be detected in the substance of the liver, spleen, kidneys, or lungs. The mucous membrane of the small and large intestines appeared to be normal. There was no recognisable evidence of the occurrence of any extensive desquamation of epithelial elements, and the tissues of the intestinal wall were free from any signs of invasion by commas.

Experiment 2.

A healthy female guinea-pig weighing 868 grammes was inoculated by the subcutaneous injection of about 0·5 c. c. of sterilised 0·8% salt solution full of commas. These were obtained from a pure tube cultivation derived from the primary tube cultivation of the cardiac blood of the previous animal. The operation was performed on the 17th February and was carried out with the same antiseptic precautions as in the previous case. The syringe employed was a different one. On the morning of the 18th the animal aborted, producing three immature young, and when first seen it was slightly lame. At 4-30 P.M. it was lame, its respirations were between 90 and 100 per minute, it was inclined to lie still and its eyes were somewhat sunken.

It died during the night, and the body was examined at 11-45 A.M. on the 19th February.

The weights of the principal abdominal and thoracic organs were as follows:—

Liver	29·42 grammes.
Spleen	0·52 „
Right kidney	2·6 „
Left „	2·55 „
Right lung	3·52 „
Left „	2·55 „
Heart	3·87 „

The appearances generally were similar to those in the previous case. There was subcutaneous sanguineous effusion in the site of injection, which, as in the previous case, was in the inner aspect of the left thigh, and extending thence over the lower half of the abdomen on both sides.

The peritoneal surface was covered by a thin, sticky, transparent secretion which caused the folds of the intestine to adhere to one another. The small intestine was almost empty, and the surface of its mucous membrane was very moist. The cæcum contained material like that in the previous case. The upper portion of the colon was empty, but towards the lower end there were a few formed fæcal pellets. The liver was pale colored and the gall-bladder full of pale, watery, yellowish fluid. The spleen was dark red. The kidneys were firm in texture, and normal in color. The lungs were pale, save towards the lower part of the left one, where some patches of congestion were present. The right side of the heart was gorged with blood. Preparations were made at once of the subcutaneous sanguineous effusion, the peritoneal secretion, the cardiac blood, and the intestinal contents.

The subcutaneous effusion was full of commas. The peritoneal secretion also contained an abundance of them. None could be recognised in the cardiac blood. The mucus from the ileum contained numerous disintegrating epithelial elements. Commas were evenly sprinkled through it, but were not present in such great numbers as in the previous case. The contents of the large intestine resembled those in the previous case in general character. Cultivations gave the following results:—

1. A tube cultivation of the peritoneal secretion gave an abundant and very rapidly growing crop of pure commas.

2. All three punctures in a tube inoculated with cardiac blood as in the previous case yielded pure crops of commas.

3. A plate cultivation of the iliac mucus yielded an almost pure crop consisting of innumerable, small, commatous colonies.

The microscopic examination of the tissues and organs also gave results similar to those in the previous case. As in it, there was no evidence of the presence of any schizomycetes in the substance of the intestinal walls. There were, however, appearances indicative of a greater tendency to epithelial desquama-

tion—appearances quite corresponding with the comparatively large numbers of detached elements present in the mucus.

Experiment 3.

A healthy guinea-pig weighing 650 grammes was subcutaneously inoculated with a drop of sterilised 0·8 salt solution full of commas belonging to a secondary tube cultivation of pure commas derived from the iliac mucus of the previous case. The operation was performed at noon on the 23rd February. The animal was apparently unaffected, but a considerable loss of weight occurred, as on the 26th February it weighed only 619 grammes. On the 1st March its weight had increased to 629 grammes, and it was then inoculated subcutaneously in the right thigh with 0·33 c. c. of material derived from a tertiary tube of a week's growth of commas derived from the iliac mucus in Experiment 2. The animal appeared to be unaffected, but a fresh considerable loss of weight occurred, so that on the 4th March it only weighed 595 grammes. A fresh inoculation was then carried out, 1 c. c. of fluid full of commas from a tube cultivation derived from the peritoneal secretion of the guinea-pig of Experiment 2, being injected into the subcutaneous tissue of the abdominal region. No result followed, and on the 8th March the body weight had gone up to 620 grammes.

The animal was then killed and the body examined with the following results:—

Weights of various organs.

Liver	30·11	grammes.
Spleen	0·7	„
Right kidney	2·38	„
Left „	2·47	„
Right lung	1·68	„
Left „	1·41	„
Heart	3·75	„

On removing the skin there were slight evidences of a limited amount of inflammatory effusion in the sites of the two later injections in the right groin and anterior abdominal surface.

The peritoneal surface was smooth, moist, and entirely free from the sticky secretion present in the two previous cases. The stomach was full of undigested food. The small intestine contained soft greenish-brown food substance. Peyer's patches were conspicuous and prominent on the mucous surface. The cæcum was full of rather pale, greenish-brown pulp, and the colon throughout contained normal fæcal pellets in its pouches. The liver was soft in texture and very full of blood. The gall-bladder contained pale-yellow bile. The kidneys were full of blood. The spleen was dark red. The lungs collapsed freely and were then pale throughout.

Fresh preparations of the remains of subcutaneous effusion and of the peritoneal secretion and blood failed to show any commas, and tube cultivations of cardiac blood, of the subcutaneous effusion and the peritoneal secretion, did not produce any.

Experiment 4.

A healthy guinea-pig inoculated on the 8th March. The weight of the animal was 776 grammes, and 1 c. c. of fluid full of commas from a pure tube cultivation of the original choleraic commas of forty-eight hours' growth was injected subcutaneously in the left thigh.

No conspicuous results followed, but as usual there was a considerable loss of weight. On the 12th March, when the weight was 700 grammes, about 1 c. c. of fluid full of commas belonging to the series of cultivation of the original choleraic material was injected into the peritoneal cavity. No apparent effects were produced and the animal was accordingly killed on the 15th March.

The total body weight was then 720 grammes, and the weights of various organs were as follows:—

Liver	37·07 grammes.
Spleen	0·8 „
Right kidney	2·4 „
Left „	2·37 „
Right lung	2·77 „
Left „	2·02 „
Heart	4·2 „

There was a large amount of sanguineous, subcutaneous effusion spreading upwards over the lower part of the abdomen from the site of original inoculation in the left groin.

The peritoneal surface was normal and devoid of any sticky secretion or signs of inflammatory action. The stomach was full of undigested food. The small intestine presented normal appearances. The cæcum and upper portion of the colon were full of soft, greenish-grey, pulpy matter, and the lower portion of the colon contained normal, formed fæcal pellets.

Fresh preparations of the subcutaneous effusion, of the peritoneal secretion, of cardiac blood, and of the contents of the intestinal tube, were examined. Unequivocal commas were absent alike from the subcutaneous effusion, the peritoneal secretion, and the cardiac blood. The intestinal contents presented normal microscopic features, and, especially as regards those related to the large intestine, contained an abundance of the commatous forms normally occurring in them in health. Tube cultivations of the subcutaneous effusion, the peritoneal secretion, and the cardiac blood, agreed in failing to yield any commas. A similar result followed in the case of plate cultivations of materials derived from the contents of the ileum, cæcum, and colon. A very extensive series of preparations were made from these, and numerous successive cultivations were carried out, but the results were merely those characteristic of cultivations of the normal

intestinal contents. In not a single case could any growth of commas be detected among the comparatively limited number of colonies of any kind which were developed.

Experiment 5.

A small healthy guinea-pig weighing 300 grammes was inoculated with about 1 c. c. of fluid containing commas from a cultivation of the original choleraic series of six days' duration. The material was injected into the subcutaneous tissue of the inner surface of the left thigh, and all the details of the operation were carried out as usual. On the following day the animal was lively and apparently quite unaffected. On the 20th of March, 48 hours after the operation, it was in the same condition. On the following morning, however, it was decidedly affected. The abdomen appeared to be somewhat swollen and it was suffering from diarrhœa. It died during the course of the following night, and the body was examined at 10 A.M. on the 22nd.

The body was perfectly fresh and *post-mortem* rigidity was well marked. The total weight of the body was 263 grammes. The weights of the principal thoracic and abdominal organs were as follows:—

Liver	15·22 grammes.
Spleen	0·6 „
Right kidney	1·25 „
Left „	1·3 „
Right lung	1·7 „
Left „	1·35 „
Heart	1·8 „

On removing the skin the subcutaneous tissue over the entire anterior surface of the abdomen and thorax was found to be deeply congested, and masses of sanguineous effusion were present in the groins and axillæ. The peritoneal cavity contained no fluid, nor were any defined adhesions present, but the surface was everywhere covered by a thin layer of sticky material which caused the loops of the intestine to adhere to one another where in contact. The parietal stratum was pinkish and slightly congested. The small intestines were full throughout of a watery fluid full of fine whitish or yellowish flocculi. In the ileum the accumulation of fluid was so considerable as to cause a certain amount of actual distension. The intestinal walls were pale, and, on laying open the canal, they were manifestly abnormally thin. The stomach contained fluid like that in the small intestine. The cæcum was half full of thick, frothy fluid of a pale greenish-grey color. Similar fluid was present in the upper part of the colon, but in the lower part a few scattered, more or less formed, pale pellets were present.

The liver and kidneys were somewhat pale. The spleen was relatively rather large and of a dull red color. The lungs appeared to be normal. The right half of the heart was full of blood.

Immediate examinations were made of the subcutaneous effusion, the peritoneal secretion, the cardiac blood, and the intestinal contents. The subcutaneous effusion was full of characteristic commas. The peritoneal secretion was also crowded with them. Many of them here were of large size and abnormal form, many of these being evidently connected with the process of multiplication by longitudinal division—using this term as applicable to that form of division, first pointed out by Dr. Klein, in which there is a tendency to the assumption of a circular figure preparatory to division (*vide* Plate I, Fig. 4). The cardiac blood failed to show any recognisable commas.

The contents of the small intestine were crowded with desquamated epithelium in the form of isolated cells and masses of adherent ones. In the fluid from the ileum commas of characteristic form were present in innumerable numbers, and so few schizomycetes of any other kind accompanied them that the material might roughly be described as representing a pure cultivation of commas. In the contents of the duodenum numerous commas were also present, but they were here associated with great numbers of large, straight, apparently putrefactive bacilli.

The contents of the cæcum were full of desquamated epithelium and contained numerous characteristic commas as well as larger commatous and spirilloid bodies similar to those present under normal circumstances.

Cultivations were carried out with the following results:—

1. A tube cultivation of cardiac blood with three distinct punctures gave a pure crop of commas around each puncture.
2. A tube cultivation of the peritoneal secretion gave a pure crop of commas.
3, 4. Two plate cultivations of the contents of the ileum yielded practically pure growths consisting of innumerable colonies of commas. Only one or two colonies of any other kind appeared in either plate.

Hardened specimens of the liver, spleen, kidneys, lungs, and large and small intestines were subsequently examined. The tissues of the liver, spleen, kidneys, and lungs were apparently free of invasion by commas or any other form of schizomycete organisms. Sections of the intestines showed absolute desquamation of the epithelial stratum (*vide* Plate I, Figs. 1-2). The subjacent adenoid tissue appeared to be everywhere completely laid bare, save in one or two rare instances where the deeper extremity of a tubular gland retained some traces of epithelium. No accumulation of commas within the adenoid tissue or the deeper strata could be discovered, and, indeed, hardly any unequivocal commas could be detected amongst the scattered bacilli which were in some places present in the substance of the exposed adenoid tissue.

We have here five cases of the subcutaneous injection of choleraic commas in three of which fatal results ensued. There can, I believe, be no reasonable doubt that the commas exerted a specific pathogenic influence in these cases. The general uniformity of the lesions present in all of them, together with the fact of the general systemic diffusion of the commas, appears to be conclusive in regard to this point.

The results are such as would appear to prove conclusively that subcutaneous injections of commatous media are not unattended with possible risks. When, however, we come to consider the matter further, we encounter two questions calling for separate consideration. These are, 1st: How far are these results confirmatory in a belief in the causative relation of the comma-bacilli to cholera? and, 2nd: How are we to account for the exemption of two of the animals experimented with?

The fact that the choleraic commas are under certain circumstances pathogenic is one thing, that they are the efficient cause of cholera is quite another. In dealing with the symptoms manifesting themselves in any such experiments as the above, we are at the outset encountered by doubts as to the extent to which we are entitled to expect any exact uniformity of symptoms in animals of unlike nature and habits, and how far we are justified in tracing parallelisms in symptoms of differing characters. The same holds good, to a certain extent at all events, in regard to the lesions revealed by *post-mortem* examinations.

Allowing all this due weight, there were apparently in these cases of disease following the subcutaneous introduction of choleraic commas into the system certain points of similarity to, or even of close agreement with, cases of cholera. So far as the symptoms are concerned, there was in the first case unquestionably considerable depression of surface temperature with frequent muscular twitchings, if not actual cramps, and, both in it and the next one, there was marked sinking of the eyeballs. In the third case, on the other hand, there was distinct diarrhœa. In all of them, as in cases of cholera, comma-bacilli were present in large numbers in the contents of the intestinal canal. The mere presence of curved bacilli would, of course, have been of no special significance, as such bodies are unequivocally present in considerable numbers within the intestines of healthy guinea-pigs. But while such commas, in so far as my experience goes, are incapable of cultivation in the agar-agar or gelatine media favourable to the growth of choleraic commas, those present in these cases are readily cultivable, and the pure cultivations of them in various media are identical in macroscopic and microscopic characters with choleraic ones. In two cases, moreover, desquamation of the epithelial lining of the intestinal canal formed a conspicuous feature, and although in one of them the process had certainly not advanced very far, in the other it had taken place as completely as it ever does even in those cases of cholera in which it attains its maximum development. Other minor points of resemblance are to be found in the great loss in body weight quite apart from any diarrhœa, and in the absence of any direct evidence of the presence of commas in the liver, kidneys, spleen, or

blood. The condition of the serous surfaces of the peritoneal cavity was also so far similar to that present in cholera in that they were covered with a thin layer of peculiar adhesive secretion.

On the other hand, this secretion is sharply defined from that normal to cases of cholera owing to the presence of abundance of commas in it. The results of cultivations of the cardiac blood, moreover, differ from those in cholera, as, although no commas were ever recognisable in the fresh blood, cultivations always gave pure crops of such bodies. In regard to this point, however, it is just possible that the commas were really derived from the serous surface of the pericardium and not from the interior of the heart. The materials for cultivation were always obtained by passing the end of a freshly drawn and hermetically sealed capillary pipetti through the walls of the auricle and subsequently breaking off the closed lip within the cardiac cavity. Now, as the external surface of the heart was in no case disinfected previously, it remains possible that the results of the cultivation were due to commas derived from the pericardium and adhering to the exterior of the pipette. The extensive subcutaneous effusion of sanguineous material full of commas which formed such a characteristic feature in all three cases is unlike any phenomenon normal to cases of cholera. The condition of the spleen, too, is, if anything, rather adverse to the acceptation of the choleraic nature of the affection, as there appeared to be a decided tendency to splenic enlargement in two of the cases, at all events, and such a condition is suggestive rather of septicæmia than of cholera.

Taking everything into consideration, however, there yet appears to be a considerable probability that the disease in these cases was in many ways closely related to cholera. The points of agreement between the two morbid conditions seem to me to be very noteworthy, and the deviations may possibly be ascribable to the nature of the animals experimented with, the quantity of the pathogenic medium introduced, and the site of its introduction.

One thing is very clearly demonstrated by the results of these experiments, and that is that the sites in an organism in which pathogenic schizomycetes are ultimately to be encountered in greatest abundance by no means necessarily correspond with those of primary invasion of the system. In all these cases comma-bacilli of identical nature with those originally introduced were ultimately present in great, and in one case in excessive abundance, within the cavity of the intestinal tube, whilst in all cases alike the site of primary invasion was the subcutaneous tissue.

It must, I think, be granted that the commas present within the intestinal cavity were the descendants of those which were introduced into the subcutaneous tissue, and, from the abundance of similar bodies present in the peritoneal secretion, it would appear that the path which they followed in their systemic diffusion was from the subcutaneous lymphatic spaces to the great peritoneal one, and thence by direct penetration of the walls of the intestinal tube to its cavity.

All direct evidence of such a process of penetration is, however, wanting, for the most careful examination of numerous sections of the intestinal walls failed in every instance to show any evidence of special accumulations of commas in their thickness. It is, of course, possible that the period of transit may have preceded that at which death took place, or that transference only occurred in the case of a very few commas which subsequently by processes of multiplication gave origin to the multitudes ultimately present within the cavity of the intestinal canal. There remains, further, the possibility that the transit may have taken place, not by a process of general diffusion, but by the agency of special carrier-protoplasts, which, after taking up the commas by ingestion while within the peritoneal cavity, subsequently traversed the walls of the intestinal tube and died within its cavity, freeing their bacillar contents in a condition fit for processes of rapid growth and multiplication. That such a process of transit actually did occur, to some extent at all events, in these cases is rendered probable by the phenomena presented by the iliac mucus in Experiment 1. In this, as previously mentioned, numerous bodies, similar to those pointed out by Drs. Klein and Gibbes as occurring in choleraic media, were present. These consisted of large nuclear bodies, with more or less evident remains of cell protoplasm around them, in which numbers of small bacilli were present. These were, however, in this instance unequivocally minute commas and not straight bacilli as in the choleraic media. (Plate I, fig. 3.) The results of the experiments in any case appear definitely to determine that by some means or other actual transit of schizomycete organisms from the tissues outside the intestinal tube to the interior of it may take place so as to give rise to conspicuous effects there without it being possible to obtain any direct evidence of the occurrence of the process.

But another important fact is established by these experiments for all those who assume that the choleraic commas are the actual cause of cholera. The experiments clearly show that pathogenic effects may arise as the result of the access of these bacilli to the lymphatic system, and that therefore the site of primary infection in cases of cholera need not necessarily be the digestive tract. It may, of course, be urged that spreading lymphatic invasion, such as was present in these cases, is not a phenomenon normal to cholera, and that it is therefore one aside of the question as regards the invasion of the system in that disease. This objection, however, ceases to carry any weight when we recollect that as an ultimate outcome of this lymphatic invasion we have the access of commas to the interior of the intestinal canal and their extensive multiplication within it; for, even granting that the true choleraic condition is only initiated by such processes, the fact remains that they may be due, not to direct invasion of the interior of the digestive tract by the ingestion of materials from the external world, but to invasion from the surrounding tissues.

The question regarding the cause of the immunity of two of the animals experimented with must in the meantime remain a subject for conjecture only. The immunity must clearly have been due either to special conditions in the

inoculated animals or to special conditions in the material which was introduced into their system. If the phenomenon were due to any subjective peculiarity in the animals there was, at all events, no direct evidence of its existence, unless we are to regard the fact that the exempt animals were somewhat larger than those in two of the fatal cases and were not pregnant as the animal in the third fatal case was, as equivalent to this. The actual weights of the animals and the amount of commatous fluid injected in each case are shown below:—

No. of Experiment.	Result.	Body Weight.	Fluid injected.
1.	Fatal.	482	1·0 c. c.
2.	Do.	868	0·5 c. c.
3.	Nothing	650	1 drop.
		629	0·33 c. c.
		595	1·0 c. c.
4.	Do.	776	1·0 c. c.
		700	1·0 c. c.
5.	Fatal.	300	1. c. c.

The above figures certainly show that, leaving the case of the pregnant animal out of consideration, the relative amount of commatous material introduced at any one time was higher in the animals which suffered than in those which remained exempt. The numbers which have to be dealt with are, however, too few to form the basis for any definite conclusion, and the matter clearly requires further experimental investigation.

In reference to the question of the possible dependence of the exemption on special quality of the injected material, it must be pointed out primarily that there certainly could not have been any permanent change or deterioration, for the two cases of exemption occurred not at the close of the series of experiments, but between the two first and the last fatal case in which the material employed belonged to a cultivation of later date than those which failed to exhibit pathogenic properties. The possibility that the exemption was due to any peculiarity in the substratum capable either of directly modifying the characters of the bacilli or of modifying the nature of the decomposition products connected with their growth, is also excluded by the fact that the substratum connected with the last fatal case was identical with that related to the two cases of exemption. In other words, the agar-agar meat-juice gelatine in the cultivations related to the two cases of exemption and to the last fatal case belonged to one batch of tubes.

The only hypothesis remaining, then, on the assumption that the exemption was due to peculiarities in the material injected, is that this presented temporary differences in the two classes of cases—that in those cases where pathogenic phenomena manifested themselves the bacilli were either more resistent and adaptible to their new site, or had manufactured special decomposition products, or specially large amounts of decomposition products favouring their invasion of the host-organism. In regard to this point also we have unfortunately not sufficient data to enable us to arrive at any definite decision.

The only phenomenon which was in any way noteworthy in reference to it is that, in all the cases in which pathogenic results presented themselves, the cultivations from which the materials for injection were obtained had very conspicuously passed on into the stage characterised by the occurrence of vacuolation and other deviations from the normal form in the commas, and that in one, at all events, of the cases of exemption, the material belonged to a very recent cultivation in which such phenomena had not begun to manifest themselves.

CALCUTTA;
The 16th April 1886.

On the Life History of a new Æcidium on Strobilanthes dalhousianus, *Clarke.*

BY

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(*With Plates II and III.*)

I.—General characters of the Æcidium as it occurs on Strobilanthes dalhousianus, with description of experiments.

For some time past I had endeavoured by numerous experiments to trace the life history of an æcidium which occurs very abundantly in Simla on *Strobilanthes dalhousianus, CLARKE*, a common member here of the Acanthaceæ. One so abundant appeared to lend itself readily to discovery; but side by side with this one so many others were met with on various hosts, and so many teleutosporic forms were found both on grasses and other plants, that I soon found myself surrounded by greater difficulties than I had anticipated. I was especially perplexed by the occurrence of equally common æcidia on *Valeriana Wallichii, D.C.*, and *Urtica parviflora, ROXB.**—plants which are generally found growing in company with Strobilanthes. It is true that the æcidium on Valeriana does not appear simultaneously with those on the other two hosts, appearing earlier in the season; but a short interval only intervenes between its disappearance and the appearance of the remaining two, and even this interval is bridged over by the occurrence of other æcidia on other plants (notably on the violet and a species of Umbelliferæ). Moreover, each of these three plants bears teleutosporic pustules long after the æcidium-bearing fungus on it has died out. At first, then, it appeared probable that these teleutosporic forms were all interrelated; but I have now satisfied myself by numerous experiments that they are not related in any way to the three æcidia above referred to. In another paper which follows I have described the life history of the Nettle æcidium, so that that of the Valeriana æcidium alone of the three still remains a mystery.

During the last days of March of the present year, I noticed for the first time that the dry leaves of *Pollinia nuda, TRIN*,† produced during the previous year, and still adherent to their dried stalks, bore numerous teleutosporic pustules. I had come up to Simla earlier in the spring than usual, and

* See another paper which follows.

† I am indebted to Mr. Duthie, Superintendent of the Saharanpur Botanical Garden, for the determination of this grass.

the season itself was unusually backward, so that a fresh spring vegetation had not yet sprung up to hide these spore-laden dry grass leaves. I placed some of these teleutospores on a few young leaves of one branch of a Strobilanthes plant I had gathered during the previous autumn, and which I had since kept in my verandah. In nature these plants were still without leaves thus early in spring, and but for the forced plants I had in my possession, collected and housed during the previous year, I should have had no opportunity then of ascertaining whether these teleutospores were related to the æcidium in question. Most of these leaves became infected, and as soon as this indication was afforded of the further life history of the Strobilanthes æcidium, numerous other experiments were instituted, many of which are detailed below, and which I think prove beyond doubt the genetic relationship between the puccinia-bearing fungus on Pollinia and the æcidial parasite on *Strobilanthes*.

The method of inoculation* I pursued was usually as follows, and, unless otherwise stated, all the following experiments were made in this way. Into a few drops of distilled water held in a watch-glass, a large number of teleutospores scraped off their beds were placed. This water was then applied by a clean glass-rod or other instrument to the leaves or other parts which it was desired to infect.

But before proceeding to a description of these experiments, it is desirable to note the general characters of the æcidium as it occurs on Strobilanthes.

The affection is especially prevalent on young plants some time before flowering. The leaves present from one to a dozen or more circular discoloured patches on their blades, measuring on an average 4—5 m.m. in diameter, though some were 13—14 m.m.; and these are usully bulged downwards with a concavity above (fig. 1). These patches, as seen on the upper surface of the leaf, are yellowish green; but as they increase in size, their centres become more purely yellow, whilst the margins only remain greenish yellow. On the upper surfaces a few minute points may be seen which are spermogonia. The lower surfaces of these patches, when young, are rosy, flesh-coloured or pale purplish, becoming yellow when the æcidia are ripe. The affection is mostly confined to the lamina of the leaf, and but rarely occurs on the petiole in nature, though, in artificial inoculations, I succeeded in infecting the petioles and even the stem on several occasions (fig. 8).

Experiment I.—This experiment has just been alluded to. The branch referred to as having been first inoculated had eleven new leaves, and spores were placed upon each of these on 1st April. On the 19th two leaves appeared to have become infected, for they presented paled spots, although as yet there were no indications of any fructification. Up to this date the plant, which was

* I shall frequently make use of this word in the following pages, meaning simply the application of spores to the surfaces of the plants, although I am aware that it is strictly incorrect to employ it in this sense; but as its use will save frequent paraphrasis, I hope it may be excused, just as with much greater indulgence the word *vaccination* is permitted to include operations not covered by the literal meaning of the word.

in a pot, was kept uncovered in my laboratory; but on the 19th it was protected still further by being placed under a glass shade. On the 23rd April nine leaves in all displayed in the same paling in spots or patches. In most cases each leaf presented several paled areas, and minute, yellowish, differentiated points could now readily be detected in several of these, which promised to develope into mature spermogonia. The anticipation was fulfilled a few days later when these points developed into minute pustules with pellucid summits, and microscopical examination left no further doubt as to their being mature spermogonia. On the 3rd May, several ripe æcidia were found in most invaded areas with well-developed æcidiospores.

Experiment II.—On the 19th April, when in the course of my first experiment it appeared probable that these teleutospores had produced a mycelium in Strobilanthes, I inoculated all the leaves of five other branches which this plant bore with the same stock of spores I had collected on 30th March, and part of which I had used in Experiment I. The plant was then placed under a glass shade and kept in my laboratory. On the 24th April, only two leaves appeared to have become attacked with paling, bulging, and slight thickening, of the blade tissues. Thinking that my spore material might have become injured in some way by keeping, I re-inoculated all the leaves which remained unaffected with freshly-collected spores on 25th April. The weather was now warmer and the leaves were attacked more quickly; for, on the 28th April, the leaves on the five branches were in the following condition: On one branch five of ten leaves were distinctly affected; on the second, which had nine leaves, none were affected; on the third, three of four leaves were affected; on the fourth, one of four leaves was affected; and on the fifth, two of six leaves were affected. The usual course of development was run through in all cases, and during the second week of May several infected areas produced ripe æcidia. The æcidiospores from these germinated readily, one of which is represented in figure 10, after having been twenty-four hours in water. As this plant was kept throughout the experiment in my laboratory under a glass shade, there was no possibility of the infection having occurred from accidental causes from without; and, moreover, throughout all my experiments, all nature acted as a control experiment, for I did not meet with affected plants in nature this year until the 15th July, although I watched constantly and carefully for its first appearance. But the artificial conditions under which this plant was placed were evidently not favourable for the complete and robust development of the fungus, as æcidial cups were not produced on the affected areas so abundantly as they are in nature. The atmosphere around it was probably too moist, and the light not sufficiently intense to keep the host in full vigour.

Experiment III.—On 5th May, two small branches were cut off from another plant kept in my verandah since the previous year, and the stems placed in water. The leaves were young, fresh, and quite normal in appearance. Upon the leaves of one (A) spores collected the same day were placed on their

under surfaces, and upon those of the other (B) upon their upper surfaces, and they were then kept under a glass shade in my laboratory. Unfortunately all the leaves of (A) died early without any sign of infection. On the 17th May, three of five leaves on one shoot of (B) and five of eight on the other shoot showed distinct foci of infection with young spermogonia already developed. On the 21st, of the ten leaves still remaining (three had withered and fallen off), all were attacked, five showing single centres of infection, three with three centres each, one with four centres, and one with five centres, of infection. The leaves withered before any æcidia were formed.

Experiment IV.—Two young plants of Strobilanthes, (A) and (B), got from nature, and which appeared to be quite free from any affection, were placed in pots. On 11th May the leaves of these were inoculated with teleutospores collected the previous day. The leaves of (A) borne on five shoots were inoculated on the upper surfaces; and those of (B), which had four shoots, were inoculated on their under surfaces. They were then kept for some days under a glass shade in my laboratory. On the 4th May, discoloured spots were observed on a few leaves of each, but fewer on (A) than on (B). On the 3rd June, after my return from a short absence, (A) had 46 leaves, of which 42 presented distinct signs of infection with numerous spermogonia, and a few æcidia ready to burst, whilst (B) had 41 leaves, of which 30 were affected, but as yet without æcidia. There was thus little difference displayed in the attack of the leaves from the upper and from the lower surfaces: those attacked from above were a little more largely and more quickly affected than those attacked from below. Later on, both plants produced an abundant crop of æcidia with well-developed æcidospores which germinated freely in growing cells, and some of which were used in infecting Stipa as described in Experiment X.

Experiment V.—The same stock of teleutospores used in Experiment IV were indiscriminately applied the same day to several leaves of a large plant I had kept in a hot-house, and which had larger leaves than any I had yet experimented with. This plant was potted during the previous autumn and, after inoculation, remained in the hot-house, where it grew luxuriantly. A large number of these leaves became infected, and on 12th June I noted that several of the affected areas showed the peculiar rosy colour below observed usually in nature, and which had not been observed in my previous experiments. The course of invasion in this case pursued a more natural course than in any of the above experiments, which I attribute to the plant having been kept under more natural conditions, to only a few of the many leaves it bore having been inoculated, and to these having been inoculated at a more advanced stage of development, and more nearly approaching the age at which leaves in nature are usually attacked. Figure 1 represents one of these leaves drawn towards the end of June.

Experiment VI.—The terminal end of a small shoot containing four leaves was cut off, and the cut end placed under water. The leaves were inoculated

on 8th May, and of these three became affected, producing a number of spermogonia. But the leaves withered before the production of æcidia.

Experiment VII.—Some seeds collected the previous autumn were sown in pots on 1st April. These produced a large number of seedlings in each pot. On 4th June, twenty young delicate leaves (ten pairs) were inoculated with teleutospores, gathered on 26th March and preserved in ordinary botanical drying-paper. At the same time some of these spores were placed in a growing cell, in order to ascertain whether they had retained power to germinate. They did not germinate readily, so twenty-five leaves were re-inoculated on the 6th June with spores collected the same day. The pot was then kept in my laboratory under a glass shade. On the 13th one leaf appeared to have become infected, presenting a yellowish point. On the 14th three leaves appeared to have become infected. On the 16th there was no longer any doubt as to the success of the experiment, as no less than ten leaves were affected, including one cotyledonary leaf. On 20th twenty were affected, some showing ripe spermogonia. On 26th I counted twenty-four affected leaves and many with ripe spermogonia, though as yet no æcidia had appeared. The plants were now transferred to a hot-house. Here they grew well, and on 1st July numerous young æcidia could be detected under the epidermis. On 4th July many of these had ripened and burst, and were filled with spores.

Experiment VIII.—Seedlings raised, as described in Experiment VII, in another pot, had pieces of the grass bearing teleutospore beds laid upon twenty leaves on 6th June. The plants were then kept in my room under a glass case. On 16th only one showed signs of attack; on 20th two, and on 26th only four, and these had developed spermogonia. The pot was now transferred to a hot-house. On 4th July I observed that eleven leaves were attacked. Several of these bore ripe æcidia, though not so many as in Experiment VII.

Experiment IX.—Five separate leaves were cut off and placed in a moist chamber on 7th June. Three of them were smeared on their under surfaces with some water from a watch-glass, into which teleutospores scraped off their beds had been placed 24 hours previously, and which microscopic examination showed to contain a number of sporidia, whilst on two of them this was placed on their upper surfaces. All those inoculated on the under surface, and one inoculated on the upper surface, became infected, producing ripe spermogonia, but withering before the production of æcidia.

In addition to these, several other experiments were made which might be quoted; but as I think those I have just described suffice to establish the fact that the puccinia spores occurring on Pollinia, when placed upon leaves of Strobilanthes, produce there an æcidium-bearing fungus, it is needless to adduce further evidence. I will, therefore, now proceed to describe the general characters of the fungus as it occurs in Pollinia, together with some experimental evidence to show that this is produced by æcidiospores from Strobilanthes, and then give the microscopic characters of the fungus as a whole.

II.—General characters of the fungus as it occurs on Pollinia nuda, with description of experiments.

Both uredo and teleutospore pustules occur almost exclusively on the under surfaces of the leaves. The pustules bearing uredo spores are generally smaller than those which produce teleutospores; are of a light-brown colour, oval or shortly linear in form, and burst by a clean longitudinal rent through the epidermis. They do not occur numerously, and, indeed, appear to have become, to a certain extent, superfluous, for the same leaves which bear a few uredo sori usually contain more numerous teleutosporic pustules. There is no doubt that the same mycelium bears both forms of spores, for in each uredo pustule several teleutospores may be found, and conversely in each teleutospore pustule several uredospores may always be found. Moreover, the same capitate paraphyses occur in the beds of both, though more numerously in uredo-beds. The mycelium produces little or no discoloration of the leaf tissue. Both forms of spores are immediately germinable after ripening. The power the teleutospores have of immediate germination was surprising, as their primary function is no doubt to preserve the fungus through the winter and to reproduce it in the following summer. Their ready germination, however, favours the view that uredospores are not required by this fungus, the teleutospores assuming both a distributive and a preservative function, though its distributive function is exercised in a different channel from that employed by uredospores. Some evidence is given under Experiment XIII to show that this view is not altogether fanciful. I collected these spores for the first time on the 26th March 1886, and found that they were then also capable of immediate germination when brought under suitable conditions, although usually in nature they do not attack Strobilanthes until the end of June or the beginning of July. At this time,—namely, in early spring,—the dried leaves of the grass may be seen sometimes to contain immense numbers of these teleutospore beds. They are generally long, linear, black beds, made up evidently by the coalescence of minute circular beds which emerge singly in a row, for some such apparently longitudinal beds when examined under a lens may be seen to consist of these circular beds which have not coalesced, or have done so incompletely. Moreover, most leaves exhibit many single isolated minute circular beds. Sometimes several adjacent longitudinal beds coalesce laterally forming a broad thick bed. These spore beds exist only, so far as I have as yet observed, on the leaves and never on the stalks.

In order to prove that this parasitic fungus is on Pollinia produced by the æcidiospores from Strobilanthes, the following experiments were made. I should mention that all these experiments were made with æcidiospores obtained from the artificially-inoculated plants described above, for throughout the whole of these experiments not a single plant of Strobilanthes became affected in nature.

Experiment X.—On the 10th June, some æcidiospores from Experiment IV A were placed upon the leaves of a few haulms, which were placed with their cut ends under water and kept in my laboratory under a glass shade. On the 27th a few pustules were found on the lower surfaces of several of these leaves. Some pustules were light brown in colour, whilst others were very dark, almost black, the latter being the more numerous. They were all minute and circular or linear, and caused little or no discoloration of the tissues around them; the spores from the light-brown sori were uredospores (one of which is represented in fig. 12), whilst those from the dark beds were teleutospores, agreeing in every respect with those which had produced the æcidiospores used in this experiment. In this experiment, therefore, I had completed a whole cycle of development of this fungus in my laboratory. Both forms of spores were placed in growing cells in pure water and germinated freely in 24 hours. As the teleutospores germinated so freely, I at the same time placed some on the leaves of two seedlings raised from seed I had collected the previous autumn. Each seedling had four leaves (excluding the cotyledonary leaves), and upon each a few spores were placed on the 4th July. First indications of attack were afforded on the 11th, and on the 18th all four leaves of one seedling were affected, each bearing ripe spermogonia, whilst one leaf only of the other was similarly attacked. This, therefore, was the second generation of the æcidial fungus developed in my laboratory. This experiment also supports the view I have taken above of the distributive function of the teleutospores.

Experiment XI.—A few detached haulms of Pollinia were placed with their cut ends under water. The leaves were dusted with æcidiospores obtained from Experiment IV A on the 15th June, and then kept under a glass shade in my room. Up to the 9th July I could not detect the extrusion of any spores, although some of the leaves appeared to have become invaded by a mycelium; but on the 13th numerous small dark-brown pustules were found, which, on microscopic examination, proved to consist mainly of teleutospores with a few uredospores.

Experiment XII.—Æcidiospores from Experiment V were placed upon nine detached single leaves which were then kept in a moist chamber; but none of them became affected, and they soon withered.

Experiment XIII.—Some plants of this grass were placed in three pots, and these were then placed under the numerously affected leaves of the Strobilanthes plant described in Experiment V, and which had throughout remained in a small glass house outside, in order that they might become spontaneously affected by falling æcidiospores. On the 13th July, several leaves were found with pustules mostly containing teleutospores. But a month later I was surprised to find that new centres of attack in large numbers had appeared on the leaves of Strobilanthes other than those I had myself inoculated. These new points of attack could only have resulted from the teleutospores which I had myself caused to be produced in the leaves of Pollinia imprisoned with the Strobilanthes in the

same glass box, for the plants were protected from external influence. It might, however, be urged that my protection against external influence was not sufficient to exclude such minute bodies as sporidia. This is true, but as I have shown that newly-formed teleutospores germinate immediately after ripening, and that they are then also fully capable of at once attacking Strobilanthes (Experiment X), it is not reasonable to invite a remote cause for the continued attack of the leaves of this Strobilanthes, when a fully sufficient proximate cause for it lies at hand. With an increased production of æcidiospores, there resulted also an increased production of teleutospores, until at last, by successive reciprocal attacks, the leaves both of Strobilanthes and Pollinia presented an immense number of infected centres far more numerous than ever occurs in nature. Some idea of the extraordinary propagation of this fungus within the glass box may be gained by the following statements. Originally about 30 leaves of this Strobilanthes plant were inoculated. They produced an abundance of æcidiospores, which falling on Pollinia, produced a first crop of teleutospores. One month later still the Strobilanthes had grown greatly, throwing out new shoots and leaves. The whole plant had its leaves sprinkled uniformly over with yellow æcidial patches. I plucked one small branch of this plant, and of the 63 leaves it bore 58 were attacked in all stages of development, from commencing attack to final disintegration of old affected areas. Two of these leaves presented over 90 centres of attack. It was difficult to count the number of centres of attack on all these 58 leaves, since many centres originally distinct had coalesced, leaving little or no indication of the number of coalesced centres. But counting all *undoubtedly* separate centres of attack, I found that these leaves presented no fewer than 730 such centres!

Experiment XIV.—A plant in pot was dusted with æcidiospores from Experiment V on the 4th July, and then kept in my laboratory under a glass shade. On the 18th I noticed teleutospore pustules on several of them.

These experiments were so fully successful that I thought it needless to repeat them, more especially as here, too, all nature acted as a control upon them, since not only at the time they were made, were old teleutospores of the preceding year's growth no longer to be found, but the new leaves of the present year's growth showed no signs of attack by this fungus.

III.—Microscopic characters of the fungus on Strobilanthes dalhousianus.

A.—On the leaf.—Mycelial filaments of the usual characters ramify through the mesophyll cells between the upper and lower epidermis layers; but whilst they occur sparingly among the palisade cells, they exist in great profusion among the cells of the spongy tissue. The palisade cells are indeed, as a rule, not disturbed in arrangement; and even when ripe æcidia are formed, the bases of these may be seen pressed against these cells, pushing them aside for about half their

depth, the upper halves remaining in lateral contiguity. The mycelial filaments are colourless, measure 4 μ in diameter, and for the most part do not penetrate the mesophyll cells, though occasionally haustoria may be seen within the cells of the spongy tissue similar in character to those which exist more abundantly in other tissues, and which will be described below. Affected areas are considerably thickened, but the thickening is due almost entirely to the increased dimensions of the spongy tissue elements, although the palisade cells are also hypertrophied to some extent. The average thickness of a normal leaf varies from about 0·129 m.m. to 0·145 m.m., whilst at invaded parts the thickness averages from about 0·315 m.m. to 0·340 m.m. The following table will show roughly the parts taken by the palisade and spongy tissue cells in giving rise to this thickening:—

	Normal leaves, two measurements.		Leaves affected to spermogonial stage.	Leaves affected to æcidial formation.
Epidermis, upper	22 μ	19 μ	25 μ	25·2 μ
Palisade cells	38 μ	69 μ	57 μ	94·5 μ
Spongy tissue cells	47 μ	38 μ	208 μ	189·0 μ
Epidermis, lower	22 μ	19 μ	25 μ	31·5 μ
TOTALS	129 μ	145 μ	315 μ	340·6 μ

B.—On petioles, leaf veins, and stem.—In a young shoot, such as that represented in figure 8, there are normally about four layers of collenchyma cells under the epidermis, succeeded by several layers, of thin-walled cortical parenchyma cells. The vascular bundles are then encountered, and within these, centrally, the pith cells. When the shoot is affected, generally mycelial filaments may be seen coursing through all the tissues, excepting the vascular bundles and the epidermis. The diameter of the filaments here I observed to be very uniformly about 5 μ. In longitudinal sections the mycelium is seen to possess a pseudo-scalariform arrangement (fig. 11) with strands of hyphæ runing more or less directly up the stem united laterally by short twigs. Although mycelial filaments do not appear actually to enter the vascular bundles, they approach them very closely. These filaments are most numerous among the collenchyma cells, and here they frequently send small twigs into the cells forming haustoria. These haustoria are usually simple unbranched tubes ending freely within the cell and without any dilatation; but in some cases they are slightly branched (fig. 13). When the petioles and veins of leaves are attacked, they are usually hypertrophied. In these parts haustoria occur, especially

within the parenchymatous cells surrounding the vascular bundles (bundle sheath ?).

C.—The æcidial fructification.—The æcidia are of the usual characters (fig 9). One of average size in a leaf measured 0·315 m.m. in depth and 0·283 m.m. in width. When young and still deep in the tissues, they are surrounded by a thick-felted pseudo-tissue of convoluted hyphæ; but later, as the fructification ripens and bursts, this covering is confined to the base. The pseudoperidium consists of a single layer of flattened angular cells, measuring on an average 16·3 × 11·6 μ (fig. 2). The upper ones have their longer diameters usually coincident with the long diameter of the æcidium, whilst the lower ones are usually at right angles to these. These peridial cells are arranged in an imbricate manner, the lower edges of each overlapping the upper edges of those below.

The hymenium consists of short club-shaped basidia, which do not form a well-defined level bed as in many cases. Æcidiospores are given off from these basidia in rows without intercalary cells. The æcidiospores are irregularly round, or more usually faceted, pale orange-yellow bodies, with deciduous, minute, oval bodies often adhering to their external surfaces, exactly like those seen in the æcidiospores of the Nettle æcidium (fig. 10). The spores measure, immediately after immersion in water, 18 × 16 μ. The epispore is not readily distinguishable from the endospore, and both together form a thin envelope. They germinate readily in pure water. Some spores taken from Experiment II were placed in water in a growing cell on the 10th June, and in twenty-four hours most of them had thrown out germinal tubes which were simple and unbranched, containing the colouring matter before within the spore body. The tubes were sometimes slightly sinuous, but never branched, with an average diameter of 5 μ (fig. 10). The young æcidia in leaves are laid in the spongy tissue; in the stem they are found either beneath the collenchyma cells, between them and the cortical parenchyma, or between the collenchyma cells. These young buried æcidia are usually somewhat flattened from above downwards.

D.—The spermogonia.—The spermogonia exhibit the usual structure. They have a tuft of hairs or paraphyses at their mouths, measuring about 80 to 90 μ in length, and 3 μ in diameter at their bases, whilst the base of the whole tuft of hairs altogether measures 56 μ in diameter. A ripe spermogonium measures about 100 μ in depth by 94 μ in width. In the leaf they occur both on the upper and lower surfaces, but more frequently on the upper. In the latter situation they may be seen apparently *replacing* several palisade cells, and their bases extend downwards a little below the lower edge line of the palisade cells. On the stem they are considerably more superficial than the æcidia lying between the epidermis and the collenchyma cells.

The spermatia are oval or pyriform bodies, measuring from 5 × 2·5 μ to 6 × 3 μ. Those figured in figure 3 were obtained from a detached leaf described in Experiment IX, nine days after inoculation.

IV.—Microscopic characters of the fungus on Pollinia nuda.

The mycelium is confined to very limited areas of the leaf tissue, extending but slightly beyond the limits of the spore beds. It requires no special description, as it agrees in every respect with the usual characters of the mycelia of other fungi of like kind.

A.—Uredospores.—These are round or pear-shaped bodies given off singly from stalks, and breaking from them when ripe without any portion of the stalk remaining adherent to them. By transmitted light they are brown, and the surface of the epispore is beset with prominent tubercles. The beds on which these spores occur bear also numerous capitate paraphyses which are colourless. In each bed several teleutospores may be found. The uredospores measure, shortly after immersion in water, on an average 21·6×20·2 μ, varying from an extreme length of 24 μ to a minimum breadth of 18 μ. Uredospores obtained from Experiment I germinated freely in water, and one of these is represented in figure 12 after lying twenty-four hours in water in a growing cell. Each spore appears to have three germinal pores on its equator, but a developed germinal tube protrudes through only one, although the merest commencements or "buds" of two others may be seen in each spore. In figure 12 such a "bud" is represented in addition to the single developed germinal tube. The germinal tubes, which attain lengths of 63 μ and upwards, are colourless, and contain finely granular protoplasm, and in diameter measure from 5 to 6 μ. The empty spores still remain brown, showing that the colour of the spore is due to the colouring of the cell-walls. The cell-walls measure about 0·75 μ in thickness.

B.—Teleutospores.—These belong to the group of Puccinia, being two-celled and borne singly on stalks which remain adherent to the spores when these are scraped off their beds. The free ends of these spores are usually rounded and slightly thickened, but sometimes they are not appreciably thickened, and sometimes they are both thickened and bluntly pointed (figs. 4 and 5). By transmitted light they are brown, and each cell contains a clear vesicular space surrounded by coarsely granular matter. The epispore is studded externally with fine tubercles, and the endospore is not separately distinguishable. The whole length of the spore measures on an average 36 μ, the septum dividing it into two almost equal divisions. The breadth at the septum is about 16 μ, and the extreme breadth of the upper cell, which is usually the broader, 18 μ. The portions of adherent stalks attached to the spores measured usually twice the whole length of the spore. The spores germinated immediately in water, the upper cell usually preceding the lower in this respect. The upper promycelium emerges from the apex of the spore a little to one side; the lower from a point near the septum. The promycelia are colourless, simple long tubes, with clear watery contents, excepting at the free ends, where they are finely granular. Sometimes, however, the promycelium is short and curved (fig 5), and such forms generally produce sporidia more certainly and more quickly than the long

promycelia. The terminal ends of the promycelia containing the granular colourless matter divide into four compartments by four transverse septa (figs. 6 and 7), and from each compartment a sporidium is found at the end of a short pointed sterigma. Sometimes the sterigmata attained abnormally long dimensions, but these never, as far as my observation reached, produced sporidia. An exaggerated instance of such deformed sterigmata is shown in figure 7. The sporidia measure from $10\times6\,\mu$ to $12\times7\,\mu$ (figs. 5 and 6). I noticed that when the germination of the teleutospores was slow in cold weather, the sporidia were smaller than when germination was quicker in warmer weather. In a cultivation started on 7th April, when the temperature of the room was about 50° F., sporidia were formed on the 11th, and these measured $10\times6\,\mu$; whilst in another cultivation started on the 9th June, when the temperature of the room was about 65° F., germination ensued rapidly, sporidia were found in twenty-four hours, and these measured $12\times7\,\mu$. The sporidia germinated readily in the usual way by throwing out slender short tubes (fig. 6). I never observed the formation of secondary sporidia.

Conclusion.—Two other species of Strobilanthes occur in the neighbourhood of Simla,—namely, *S. alatus*, NEES, and *S. atropurpureus*, NEES; but although the former is very common, and may often be found growing side by side with *S. dalhousianus*, I never observed the æcidium on either of them. Both *S. alatus* and *S. dalhousianus* harbour a parasitic fungus bearing Uromyces spores during the autumn, but I have not been able to ascertain either the origin of this fungus or its future development. It apparently has no connection whatever with the æcidial fungus just described. This affection is very common indeed on *S. alatus*, and less common on *S. dalhousianus*. Inoculation experiments made on *S. dalhousianus* this year with spores collected the previous autumn were without result; but I should also mention that I could not get these spores to germinate in artificial cultivations.

I propose to call this fungus, which belongs to Winter's group of Heteropuccinia (heterœcious species),* *Æcidium Strobilanthis*, and its diagnostic characters are as follows:—

Æcidium Strobilanthis, *Barclay.*

I.—Æcidia usually hypophyllous, borne in clusters on somewhat thickened discoloured areas on leaves, seldom on petioles or young shoots. Areas greenish yellow to yellow above and pale rosy or purplish to reddish yellow below; pseudoperidium cup-shaped or cylindrical with white stellately split edges. Spermogonia hypo- and epi-phyllous with tufts of paraphyses around their mouths. Spermatia oval or pyriform, $5\times2{\cdot}5\mu$ to $6\times3\,\mu$. Æcidiospores

* "Die Pilze, Deutsch. Oest. u.d. Schweiz." (Rabenhorsts Kryptogamen Flora, I Band.)

irregularly round and usually faceted, 18×16 μ, smooth, pale orange-yellow. On Strobilanthes dalhousianus, ***Clarke.***

II.—Uredopustules, circular, oval or linear, pale brown, hypophyllous, on blades of leaves only. Spores round or pyriform, brown; epispore beset with prominent tubercles; numerous capitate paraphyses throughout spore bed. Spores 21·6×20·2 μ. On Pollinia nuda, ***Trin.***

III.—Teleutospore beds circular, oval or linear, dark brown or black, hypophyllous, on blades of leaves only. Spores produced by same mycelium producing uredospores, brown, free end rounded and slightly thickened; epispore studded with very minute tubercles. Whole length of spore 36 μ and breadth at septum 16 μ; stalk 72 μ in length. Promycelia form four sporidia, measuring 10×6 μ to 12×7 μ. On Pollinia nuda, ***Trin.***

SIMLA;

The 1st August 1886.

Æcidium Urticæ, *Schum.*, var. *Himalayense.*

BY

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(With Plates II, IV, and V.)

I was not aware, when during 1885 I traced the life history of the very striking æcidium which occurs abundantly on the nettles about Simla (Western Himalayas), that a similar, if not identical, one is well known in Europe, and had been described by Schröter.* Upon learning this I decided not to publish the results of my own observations; but, on reconsideration, I have thought that it would not be uninteresting to those interested in these fungi were I to do so, as the Himalayan variety differs in some respects from its European congener, and is of peculiar interest in regard to its geographical isolation or at any rate remoteness. The traveller on the Himalayas is frequently struck by the presence there of flowering plants very closely allied to, or even identical with, European species; but the occurrence of a similar heterœcious Uredine requiring two widely different hosts for its existence is still more astonishing.

This Himalayan variety completes its existence on *Urtica parviflora*, ROXB., and *Carex setigera*, DON, while these two hosts are usually replaced in Europe by *Urtica dioica*, *L.*, and *Carex hirta*, *L.*† *Urtica dioica* does not occur in the Himalayas so far eastwards as Simla, although it is common enough farther westwards in the Punjab and Afghanistan. The nearest allied species in Simla to *Urtica parviflora* is *Girardinia heterophylla*, DECAISNE, a large stinging nettle with cut leaves which generally grows side by side with *Urtica parviflora;* but the æcidium never occurs on it.

When *Urtica parviflora* is attacked by the fungus, very remarkable distortions and hypertrophies are caused, an extreme instance of which is represented in Fig. 1, Plate IV. The fungus attacks both leaves and stem, but is always localised, never pervading the plant generally and causing its complete destruction. When it occurs on the leaf, it is seen to have a special proneness to attack the petioles or veins, and the parts invaded by the mycelium swell so

* Cohn's Beiträge zur Biologie der Pflanzen, Band I, drittes Heft, 1875.

† In the European variety, several other species of Carex, however, may bear the uredo and teleuto spores, whilst *Urtica pilulifera* and *U. urens* may also bear the æcidia. See G. Winter, "Die Pilze Deutschlands" (Rabenhorsts Kryptogamen Flora), and Schröter, *l.c.*

greatly that the whole leaf becomes extraordinarily deformed. When the veins are attacked in their continuity, they curve while swelling very greatly with their convexities usually downwards; but when the fungus attacks the branching point of smaller veins, the most irregular contortions arise, some curving with their convexities upwards, whilst others are twisted in the opposite direction. Some idea may be gained of the extraordinary amount of hypertrophy produced by the following actual measurements. The petiole of a leaf which was monstrously hypertrophied measured 12·7 c.m. in length and 2·2 c.m. in diameter at its thickest part, and was curled round in a complete circle. When the petioles are invaded their whole tissue is uniformly hypertrophied; but when the main stem is attacked (Fig. 1, Plate IV) the mycelium is strictly localised, and the hypertrophied part appears in the form of an excrescence or well-defined tumour. Such an excrescence may or may not cause the stem to be bent towards the opposite side. An extreme amount of such bending is shown in Fig. 1, Plate IV. These tumours are often of great size; one, by no means the largest that could be found, measured 2·8 c.m. in length, 2 c.m. in breadth, and 1·2 c.m. in depth. These extraordinarily hypertrophied parts contain an abundance of nutritive starchy material, which the hill natives eat with relish, selecting them just before the æcidia ripen. It is called by them *khiri*, a cucumber, on account of the resemblance of its taste to that vegetable.

Generally, during the middle of June, the upper surfaces of the leaves of the nettle present small circular patches of pale-yellow discoloration. Each patch is usually much arched upwards with a corresponding concavity below. At this time a leaf may have from one to five such patches. The upper surfaces of these patches are densely studded with spermogonial papillæ, a few only occurring on the lower surfaces. A fortnight or three weeks later the æcidial fructification makes its appearance on the same patches, which have by this time increased in area and thickness. Now, during the earlier part of July, large thickenings and tumours occur plentifully; but as soon as the rains have well set in, these hypertrophied parts disappear, due to their rotting, mainly the result of invasion by a white silky mould. But although at this season large hypertrophies are not to be found, except in sheltered places, yet infection continues, for young leaves even then show the earliest stages of attack by the parasite. Towards the latter end of the rains, however, after the middle of August, the fungus disappears entirely, and will not be found again until the following summer. The nettle flowers towards the end of the rains (during the latter part of August), and I have in several other cases observed that when a flowering plant is attacked by an æcidium before it flowers, the fungus disappears entirely just before the flowers begin to open. For example, this is the case also with Æcidium Strobilanthis described in the preceding paper. The above seasonal characteristics of the fungus relate only to its appearance in Simla. At lower elevations it appears earlier, for during May 1884, whilst at Rampur and other

places in the Sutlej Valley at elevations of 3,500 to 4,000 ft., I found this fungus already in full æcidial fructification.

Having given a brief description of the general characters of the æcidial stage of this fungus, I will now describe some of the experiments made to show that it is produced by teleutospores from *Carex setigera*.

Experiment I.

On the 21st June 1885, some teleutospores scraped off from their beds on Carex were placed on the leaves of a nettle gathered some weeks previously from a locality in which the fungus was not observed, and which had since remained unaffected in a small glass house in my garden. About a fortnight later there were distinct evidences that the plants had become infected, as several minute yellow spots were observed on the marked leaves which had been inoculated. These spots gradually increased in size, and on the 10th July bore numerous ripe spermogonia. In the same glass house were several other nettle plants which had not been inoculated, and these as well as the unmarked leaves of the plant partially inoculated remained unattacked. The affected parts continued to increase in area and thickness, and ultimately yielded characteristic hypertrophies densely studded with ripe æcidia.

Experiment II.

A pot containing three seedlings raised from seed I had collected the previous year and sown on 29th March 1886, offered 16 leaves in all for inoculation. Upon 12 of these, scraped-off teleutospores were placed on the 6th June, and the pot was then placed in a glass house outside. On the 26th June only two leaves were affected, and no more became subsequently attacked. On the 4th July, a vein of one of these leaves was largely swollen, and under the epidermies some æcidia were observed which a few days later came to maturity.

Experiment III.

A pot containing three similar seedlings had the terminal pairs of leaves of each inoculated on the 6th June. The plants were then kept in my room under a glass shade. On the 14th three leaves showed commencing invasion, and on the 26th five of the six inoculated leaves were attacked, and some bore ripe spermogonia. The plants were not vigorous and had not grown since inoculation. They did not form æcidia.

Experiment IV.

Seven similar seedlings had their terminal pairs of leaves inoculated on the 6th June, and were then placed, like those just described, in my room under a separate glass shade. None of them became affected.

Experiment V.

Another pot also containing seven seedlings was treated in an exactly

similar way on the same day, but instead of being then kept in my room was removed to a glass house outside. On the 26th four leaves were found attacked, and on the 4th July five of the seedlings had each its pair of inoculated leaves attacked, whilst the remaining two seedlings showed each only one leaf attacked. These plants had grown more vigorously in the glass house than those similarly treated in my room (Experiments III and IV), for each plant had unfolded two additional pairs of leaves above those inoculated, and these new leaves remained quite free of the fungus. I think the small success attending Experiment III and the entire want of success in Experiment IV may therefore be ascribed to the unfavourable surrounding circumstances in their cases.

Experiment VI.

On the 7th June four leaves of a seedling were cut off, and their cut ends placed under water. These were inoculated with teleutospores which had been scraped off their beds, and which had thereafter lain 20 hours in water, by which time numerous sporidia had been formed. Three of these became infected and produced ripe spermogonia, but did not go on to the formation of æcidia.

Experiment VII.

On the 13th April some scraped-off teleutospores were placed on the leaves of a young, uprooted, vigorous nettle gathered in my garden, and then placed with its roots under water in my room under a glass shade. On the 24th I observed that infection had undoubtedly occurred with paling of the leaf tissues and bulging in all the inoculated leaves, and in one the formation of spermogonia had commenced. Later several leaves formed spermogonia, but they all withered before any æcidia were produced.

Experiment VIII.

I sowed some nettle seed gathered during the previous autumn in two pots on the 29th March. On the 17th May these pots were crowded with seedlings which had unfolded from one to two pairs of foliage leaves. They were then sprinkled with a little water into which a large number of teleutospores were introduced. On the 3rd June a great many leaves were attacked, and many had produced ripe spermogonia. Later some æcidia were produced on a few leaves, but most of the affected leaves withered early. In every case in which æcidia were produced, the veins running across the affected areas were largely swollen.

Experiment IX.

Six of these seedlings were uprooted prior to the commencement of Experiment VIII, and having been placed with their roots under water, were inoculated with the same material as was used in Experiment VIII, and then kept

under a glass shade in my room. Only one of these plants became infected, and all withered early.

In reviewing the results of these experiments, some explanation seems called for to account for the numerous cases in which infection did not follow inoculation in experiments made upon seedlings. In the first experiment made upon a fully grown plant, not only did all the inoculated leaves become attacked, but all produced numerous æcidia; whilst in the case of inoculated seedlings, even when infection resulted, the growth of the parasite often advanced only to the production of spermogonia. Two reasons may be given for the comparative insufficiency of result in these cases. The first is that the seedlings were very delicate from having been grown under artificial conditions: they were mostly elongated and etiolated, and in some cases, as in Experiment VIII, too densely crowded together. They were thus not robust plants, and could do little more than nourish themselves. In the second place, we have seen that this fungus requires considerable aid from its host for its own complete development, as the ripe æcidia are always borne after the attacked parts have swollen very considerably and amassed a large reserve of nutritive material. In nature æcidia are never found except on very considerably hypertrophied parts; hence the seedlings were unable to nourish a parasite that demanded so much of them in their enfeebled condition. These reasons, I think, will be considered sufficient to account for the several failures in my experiments with seedlings, more especially as they are indirectly supported by the complete success attending my inoculations upon vigorous and fully developed plants, many of which I have not thought it necessary to record here.

I will now proceed to describe the fungus as it occurs on *Carex setigera*. This is a very widely distributed plant in Simla. Uredo pustules may usually be found, though not abundantly, on its leaves, and sometimes on its stalks, during the time the æcidia on the nettle are ripe,—that is, generally during July. They occur mostly on the upper surface, and generally on the distal half of the leaf. They are very inconspicuous, small, brown, linear or oblong sori.

At this time, while uredo pustules were common on the green blades, teleutosporic pustules might usually still be found on the dried but still adherent leaves of the preceding year's growth at the bases of tufts of these young leaves. These pustules are also extremely inconspicuous on account of their minute size. They are black and circular, often very numerous mainly on the upper surface, but occasionally also on the lower. Towards the end of July and during August, new teleutospore beds are formed on the green blades.

I made a few attempts to produce uredo pustules on Carex leaves in my room by inoculating them with æcidiospores, but without success. The leaves withered quickly in the room. I succeeded, however, in producing very numerous pustules on tufts outside my house by dusting them with æcidiospores; but it is of course quite possible that the resulting infection may have been due to natural attack, for the plants were not protected in any way. Still, as the plants which were in a naturally sheltered nook had been dusted very profusely with æcidiospores and bore many more uredo and teleutospore pustules than are usually to be found on naturally attacked specimens, the greater probability is that the leaves in question became attacked by the æcidiospores I had dusted over them. I did not consider it necessary to make further efforts to artificially reproduce the Carex effection, as this had been done in Europe by Magnus as related by Schröter in his work already quoted.

MICROSCOPIC CHARACTERS.

General.—The enormous hypertrophy of the parts attacked by this fungus is due mainly to the increased size of the parenchyma cells, and, to a very subordinate extent, to mycelial invasion. The mycelium never occurs in masses except in the immediate neighbourhood of æcidia and spermogonia. Indeed, hyphæ in any large numbers are only to be seen to a depth of about three or four cells beneath the bases of æcidia. Elsewhere single filaments are seen coursing throughout the hypertrophied tissues. They are also to be found between the elements of the fibrovascular bundles, though sparingly. There is therefore no general separation of the parenchymatous cells. The hyphæ are very translucent (and therefore difficult to detect without staining), septate, branched, and measure on an average from 4 to 5 μ in diameter.

The mycelium penetrates some of the cells forming haustoria of the branched type. Sometimes the haustorium consists of a single slightly convoluted twig, at others it is arborescent (Figs. 4 and 5, Plate IV). They occur most abundantly within the parenchyma cells adjoining those immediately surrounding the fibrovascular bundles (bundle sheath cells), and are best seen in longitudinal sections. They occur also in other cells of the parenchyma as well as in the sheath cells themselves. The contents of these haustoria are very finely granular, and in this respect differ from the hyphæ generally, which, as already observed, are very translucent. They may also be seen in the leaf tissues, both in the spongy and palisade cells (Fig. 21, Plate II).

The hypertrophied cortical parenchyma cells are filled densely with large granules, which are apparently of the nature of starch, for they become deep blue on the addition of Schultze's solution (chlor. zinc. iod.), but they do not exhibit any traces of striation nor of hila. With Spiller's purple, these grains are sharply dis-

tinguished from other elements of the tissue by their crimson-red colour. They are also quickly soluble in water, for in sections cut in water they are generally missed, whilst they are always very abundantly present in sections cut and examined in glycerine. These granules vary very greatly in size from about 16 μ in diamater to 4 μ (Figs. 2 and 3, Plate IV). The smaller ones are more abundant than the larger, which appear to be compounded of several smaller ones (Fig. 3, Plate IV). The central hypertrophied pith cells do not contain these large grains, but with Schultze's solution a number of fine, minute, deep-blue granules may be seen throughout them.

Spermogonia.—These present the usual characters. A fully ripe one measured 0·178 m.m. in breadth and depth; a younger, still unopened one, 0·104 m.m. in both directions. They have a radiating pencil of hairs on their summits measuring about 68 μ in length. The spermatia are very minute, oval, rod-like, or often pyriform bodies, and with certain focussing present a minute black point in their centres. They vary in length from 3 to 4 μ, and are about 1 μ in breadth. The spermogonia do not exhibit the same proneness to develope in the fibrovascular tissues as do the æcidia. They occur mostly in the parenchymatous tissue of the leaf. In the spermogonial stage, and when as yet there are no signs of the formation of æcidia, the thickness of the affected portions of the leaf-blade is almost exactly double that of the normal leaf-blade. The parenchyma cells of the involved parts are filled with large granules similar to those above described.

Æcidia.—The young æcidia filled with spores but still unopened are spherical, but often slightly compressed from above, their breadth exceeding their depth a little. Such æcidia measure from 0·175 m.m. in breadth and 0·139 in depth, to 0·294 m.m. in depth and breadth. Large fully ripe and open ones measure as much as 1·061 m.m. × 0·353, and even more. The æcidia are enclosed by a peridium of a single layer of flattened cells which measure on an average 20 μ in diameter. These cells are considerably thickened on their outer sides (Fig. 9, Plate V), and with chlor. zinc. iod. become brick-red. When seen from the surface, they are polygonal (Fig. 20, Plate II). They are so arranged that the upper edge of each cell is overlapped by the long, bevelled, lower edge of the cell immediately above it. The arrangement is therefore imbricate. The internal surfaces of these cells are densely studded with characteristic tubercles. In section these cells measure 20 μ in length by 20 μ in depth, but 6 μ of the latter are due to the outer thickened wall. The cells lower down are generally about 14 μ in length. Outside the peridium is a layer of convoluted mycelial elements. The æcidiospores are given off in extremely long rows from a series of basidia arranged very regularly, their upper ends forming a level base. These basidia are on an average 40 μ in length and from 2 to 2·5 μ in diameter. They rest upon a base of convoluted fibres forming a pseudo-parenchymatous mass. This together with the basidia and the lower younger æcidiospores take carmine stains well.

With chlor. zinc. iod., the upper riper spores become greenish brown, whilst the younger spores and basidia assume a pale-sherry colour. As many as 24 spores may be counted in a detached row. They have no intercalary cells. The spores are very pale yellowish-white by reflected light, and when dry are usually faceted. In water and by transmitted light, they appear as yellow round bodies with finely granular contents. Externally they are beset with very deciduous tubercles (Fig. 6, Plate IV, and Fig. 8, Plate V). The moistened spores measured on an average 15·6 × 13·1 μ, and these measurements were very uniformly maintained. In germinating, the æcidiospores throw out curved germinal tubes, which contain pale yellow oil globules as well as clear watery parts and granular protoplasm. The tubes measured 4 μ in diameter. Frequently these æcidiospores refused to germinate, and then after lying a few days in water, the spores generally lost much of their colour, and their contents were resolved into a number of round colourless globules mostly double-contoured and of various sizes (Fig. 7, Plate IV).

Uredospores.—The uredospores are elliptical or pyriform bodies borne singly on stalks shorter than their long diameters. They measure from 19·8 × 13·5 μ to 19·2 × 12·8, and are beset externally with prominent tubercles (Fig. 10, Plate V). They are pale yellow by transmitted light, with a thick epispore beset with prominent tubercles. At the equator of the spore two germinal pores may be seen after it has lain for some hours in water. Among the uredospores some club-shaped paraphyses always occur. Placed in water these uredospores germinate readily, throwing out *one* long germinal tube which is unbranched, or with one or two very short lateral protuberances. No germinal tube issues from the other germinal pore. The germinal tubes measure 4 to 5 μ in diameter.

Teleutospores.—The teleutospores are two-celled bodies, and therefore belong to the class of Puccinia. They are dark brown and given off singly on long stalks of about 56 μ in length and 5 μ in diameter. The spores are very firmly adherent to their beds. Each compartment of the spore is generally considerably longer than it is broad, but occasionally spores may be seen in which the two compartments are nearly spherical. The upper compartment is usually much deeper in colour than the lower and more spheroidal. The lower cell usually tails off, contracting gradually towards the stalk, which is broadest where it joins the cell, narrowing gradually lower down. Hence the lower cell appears gradually to contract to the narrow diameter of the stalk (Fig. 11, Plate V). An abnormal form in which two spores are borne on a single stalk is not uncommon (Fig. 17, Plate V). The free end of the spore is generally thickened to the extent of about 6 μ and conical, and a clear vesicular space may generally be seen in each compartment. The length of the upper compartment varies from 24 to 20 μ, and its breadth from 18 to 17 μ, whilst the length of the lower cell varies from 20 to 14 μ, and its breadth from 18 to 14 μ. Among the teleutospores scraped off their beds a few uredospores may always be found, from which it may be concluded that the same mycelium produces both forms of spores. When

placed in water in a growing cell, these teleutospores germinate quickly, each compartment throwing out a colourless germinal tube or promycelium, the upper one usually preceding (Figs. 12, 13, 16, and 17, Plate V). Teleutospores are generally formed about the beginning of August, and are germinable from the time they are ripe until the following July, but thereafter they are no longer capable of germination. During August I several times collected old teleutospores from the preceding year's dried-up leaves and new ones from fresh green leaves, and placed them in growing cells in water under exactly similar conditions, and whilst the new teleutospores germinated freely, the older ones refused to do so absolutely. This power of immediate germination of teleutospores may account for the few uredospores formed, as explained in the preceding paper on Æcidium Strobilanthis.

The promycelia are sometimes short curved tubes uniformly granular in contents, or long straight tubes with the granular protoplasm partly collected at the ends and partly broken up into narrow septa or beads dividing off long clear spaces of the tube filled with fluid. Some of these long promycelia measured 0·2 to 0·4 m.m. in length and 5 to 5·5 μ in diameter, whilst the shorter curved promycelia were somewhat stouter, measuring 6 to 6·5 μ in diameter. The promycelium of the upper cell emerges from the apex of the spore body, whilst that of the lower protrudes from a point near the septum (Figs. 12, 13, 16, and 17, Plate V). The distal ends of the promycelia are divided into four compartments by transverse septa, and from each of these a sporidium is usually formed at the end of a short pointed sterigma about 12 μ in length (Figs. 14, 15, Plate V). The sporidia are oval, pyriform, or slightly kidney-shaped, measuring about 12 × 8 μ. The sporidia germinate in the usual way by throwing out a slender tube. As a rule secondary sporidia are not formed, but I once saw a secondary sporidium produced which measured 6 × 5 μ.

Conclusion.—In conclusion, I will note briefly the chief differences between the Himalayan variety of this Æcidium and its European congener. As regards the mycelium, the only difference consists in the presence of haustoria in the Indian variety, whilst they are absent in the European form. The spermogonia are somewhat larger in the Indian variety, measuring about 0·187 m.m. in width and depth against 0·100 to 0·120 m.m. in the European form. The æcidiospores of the European form measure 20 to 17 μ × 16 to 12 μ, whilst in India they are 15·6 μ × 13·1 μ; and with these differences there are also differences in the diameters of the germinal tubes, which are 5 to 6 μ in the European form and 4 μ in the Indian.

The uredo and teleutosporic pustules on European species of Carex are found on the lower surfaces of the leaves, whilst on the Indian species they occur on the upper surfaces. As in the case of æcidiospores, the Indian uredospores are smaller, measuring 19·2 to 19·8 μ long by 12·8 to 13·5 μ broad against 23 × 19 μ in the European form.

The teleutospore beds in the European form are described as in long

parellel rows, but in the Indian variety they are isolated, small, circular beds. The teleutospores in both forms are of about equal total length; but whilst in the European form the lower cell is often longer than the upper, the reverse is the case with the Himalayan variety. The stalks of the European form are about 20 μ long; in the Indian variety they are about 56 μ long. The sporidia in the European form measure $10 \times 6{\cdot}6$ μ, whilst in the Indian variety they are 12×8 μ; and whilst the former appear usually to form secondary sporidia, the latter do so very exceptionally.

PUCCINIA URTICÆ, nov. sp.

P.S.—The leaves of *Urtica parviflora* often bear numerous teleutosporic pustules during the autumn long after all trace of the æcidial affection above described has disappeared and when the plants are in flower. I have counted as many as 120 pustules on a single leaf-blade. This affection has apparently no relationship whatever with *Æcid. urticæ*, for when the spores are placed upon sound nettle leaves no result follows. It must therefore be related to some other æcidium; but in spite of numerous attempts by experimental inoculations to discover its future history, I have failed entirely to do so. The pustules vary in size from that of a pin's head to 3 m.m. in diameter. These pustules are on the under surfaces of the leaves; they are deeply convex there with a corresponding concavity above. The invaded areas are at first pale yellow both above and below, but later they become brown below, whilst above they are orange-yellow with a pale-green margin. The pustules consist of aggregations of minute, circular, prominent spore-beds. The spores are borne on long stalks, and are very firmly adherent to the leaves. They are brown with a thick epispore, thicker at the apex than elsewhere, and show no surface markings (Fig. 18*a*, Plate V). The spores vary greatly in size and shape, an average one measuring about 40 μ in extreme length, of which 18 μ belong to the upper cell and 22 μ to the lower, whilst the extreme breadth of each cell was 17 μ. An attenuated spore measured 38 μ in length by only $8{\cdot}5$ μ in width, whilst a plump spore measured 40 μ in length, the upper cell being 19 μ wide and the lower $14{\cdot}8$ μ. An abnormal double-headed spore is shown in Fig. 18 *b*, Plate V, and such spores are not rare. Some freshly-collected spores placed in water on 11th September germinated freely in 24 hours, each cell throwing out a promycelium, that from the upper cell emerging from the apex, and that from the lower cell from a point near the septum. The promycelia are usually long, straight, colourless tubes, measuring generally 8 μ in diameter at their bases, but sometimes only 6 μ, and diminishing gradually to 2 or 3 μ at their apices. My cultivations in pure water never attained to the perfect development of sporidia, but apparently the terminal ends of the promycelia divide into three compartments, from each of which one sporidium is formed measuring 12×8 μ.

SIMLA;
The 3rd August 1886.

On the Phenomenon of Gaseous Evolution from the Flowers of Ottelia alismoides.

BY

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(*With Plates VI, VII, and VIII.*)

Ottelia alismoides is a plant very abundant in the tanks in and around Calcutta, and, along with *Vallisneria spiralis*, stands out conspicuously as an important source of oxygen supply to the water. Certain peculiar phenomena connected with the gaseous evolution from the plant have not, in so far as I know, been specially described, and in the present paper I propose to give a brief account of them, founded on observations which were in greater part carried out in the early part of the year 1885. During the greater part of the time in which any plant of Ottelia is in full vigour the evolution of gas from it is not of a character to attract casual attention, as it is carried out by means of an abundant ascent of minute bubbles from the edges, and, to a slight degree, from the upper surfaces of the large, broad leaves. The great site of discharge of gas from the tissues is at this time the inferior surfaces of the leaves. The large amount of gaseous evolution actually occurring under such circumstances becomes, however, at once evident on removing a plant from its natural site and introducing it into a glass jar full of water and exposed to sunlight. A continual stream of small bubbles is then seen ascending from the foliar surfaces.

Such phenomena continue to present themselves unaltered during the period of growth of the scapes and early development of the flower-buds, but during the single day in which any flower matures and blows they are to a great extent replaced and masked by others. The gaseous evolution at this time becomes extremely conspicuous owing to the constant discharge from the flower of a stream of bubbles of such considerable size as to be readily observable whilst the plant remains in its normal site, and the flowers deeply submerged, as the majority of them are, beneath the surface of the water.

The general sequence of events in the blooming of the subaqueous flowers of Ottelia (Plate VI, Fig. 1) is as follows: The actual flower-buds, up to the day on which they expand, are completely enclosed by a continuous sheath of fused bracts, forming a tube around the elongated ovary and arching over the young calyx and

corolla. Up to this time, too, the calyx is closed, the three sepals composing it adhering to one another marginally, so that the cavity of the flower is completely separated from the surrounding water by two continuous coverings—one bracteal and one calycine. During the course of the night, previous to blowing, the apex of the bracteal sheath gives way under the pressure of the included and growing flower, and the tip of the bud then protrudes free into the water. Development now advances rapidly. In the early morning the buds are still quite closed, the sepals still being closely applied to one another and completely concealing the white petals from view. If the plant in this condition be well exposed to the access of sunshine, active discharge of large bubbles from the interior of the bud now sets in, the rate of evolution varying under various circumstances—the degree of exposure to light, the general vigour of the plant, &c. (*vide* Note A). If a closed bud in an active state of gaseous evolution be examined, it will be found that the essential organs have already matured, that an abundance of pollen is smeared on the stigmas and on the interior of the corolla at the level of the fully open anthers, and that free germination of pollen grains and passage of pollen tubes down the greater part of the length of the central stylar canals has already taken place (Plate VI, Fig. 4). Gradual opening of the calyx and protrusion of the folded petals soon occur, these processes being aided by the continuous accumulation of gas within the cavity of the flower and its intermittent discharge in the form of large bubbles passing out between the opposed margins of the sepals and petals. Unfolding of the corolla next occurs, active evolution of gas continuing as before. When once, however, the corolla is fully unfolded, the surrounding water descends into its cavity and appears to come into direct contact with the stigmas and styles. In the course of the afternoon active evolution of gas gradually diminishes, and with sunset comes to a close. The flower is at this time fully expanded and the corolla retains its pure white colour, the water apparently hardly coming into contact with it, especially externally, owing to the thick stratum of adherent bubbles of gas with which it is coated. On the following morning this gaseous coating is absent, the corolla is already brownish and macerated, and no further evolution of gas from the flower takes place on the renewed incidence of sunlight.

In studying these phenomena, one of the first questions which naturally suggests itself is whether the gas discharged by the flower is of the same quality as that evolved from the plant generally, or whether it is the product of special respiratory processes connected with the maturation of the floral organs and the occurrence of fertilisation. The large quantities of gas which are discharged permit of the ready determination of this point, and the analysis of the gases collected from the flowers and the rest of the plant separately show that the quality is in both cases alike. In both cases there is an absence of carbonic acid, and the gas consists of a mixture of atmospheric air and oxygen. The proportions of atmospheric air and oxygen in 100 volumes of the gas in one experiment where this point was specially determined were 62·42 atmospheric air to 37·58 oxygen. The next question calling

for determination is whether any special evolution of gas takes place during periods of flowering in excess of that evolved at other times. Experiments of various kinds (*vide* note B) agree in indicating that no special evolution occurs. In the first place it can be shown that in plants from which all the leaves have been carefully removed hardly any evolution of gas manifests itself through the flower. In the second place it appears that the amount of gaseous evolution from the flower varies in direct proportion to the number and size of healthy leaves on the plant. Finally, it is demonstrable that, allowing for this, the total gaseous evolution from both the flowers and general surfaces of flowering plants closely corresponds in amount with that from the general surfaces alone of non-flowering ones.

Such a peculiar phenomenon might on *a priori* grounds lead to a belief that it was connected with some important end in the life history of the plant, and on examining the subject more closely from this point of view, it becomes evident that this is actually the case.

The great end which is attained by the large liberation of gas from the floral organs is that fertilisation is securely carried out in the submerged flowers which come to maturity while far beneath the surface of the water. The majority of the strongest plants of Ottelia grow in such deep water that none of their flowers have a chance of ever reaching the surface, and, even in plants growing in shallower water, very many flowers remain permanently submerged. Were no special provision at hand a very large proportion of flowers, and all those of the most vigorous plants, would fail to produce any seed, as the pollen grains are affected by contact with water in the ordinary fashion, swelling up, bursting, and discharging their contents without germinating.

Owing, however, to the constant evolution of gases taking place in the different parts of the flower at the period of maturation of the essential organs, these are kept continuously free from contact with the surrounding water, and germination of the pollen grains and penetration of the stigmatic tissues are accordingly permitted to proceed without any obstacle.

The flowers of Ottelia, whether aerial or subaqueous, are essentially developed so as to secure self-fertilisation. The anthers and stigmas mature simultaneously and at a period when the flower is still in bud. The filaments and styles are in close apposition and are arranged alternately, and the anthers are closely applied to the stigmas in such a fashion that, as a rule, each of the anther lobes is on opening in direct relation to one of the stigmatic lobes of a separate stigma connected with a separate style.

The precise arrangement and relations of the anthers and stigmas varies naturally to some extent in different instances (*vide* Note B), but in any case the relation is one of direct apposition, and the pollen, on dehiscence of the anther lobes, is directly applied to the stigmatic surfaces in large quantities. The pollen grains, moreover, germinate at a very early period, many of them beginning to do so whilst still within the anther lobes and apart from contact with the stigmas.

The germinating tubes push their way inwards through the hairy tissue of the edges of the stigmas, to reach their smooth central surfaces, and then pass vertically down these and the stylar canal which is continuous with them. The number of pollen grains which have germinated *in situ* within the anther lobes is sometimes so great as to cause the anthers to adhere to the stigmas, and the mass of tubes traversing the stylar canal is so great as to form a thread readily distinguishable by the unaided eye if the style be carefully divided and the distal and proximal portions gently separated from one another (*vide* Note D).

All these processes of maturation of anthers and stigmas, germination of pollen grains, and ingrowth of pollen tubes, take place ere the flower-bud has expanded, and at a time when in submerged specimens its cavity is constantly occupied by gas distending the floral chamber and discharging its excess in the form of a continuous stream of bubbles, which force their way out between the margins of the floral envelopes.

The evolution of gas seems to take place from all parts of the flower, but mainly from the internal surfaces of the sepals, the external surface of the petals, and the tissue of the upper extremity of the centre of the ovary at the bases of the styles and continuous with the very open tissue which holds the central extremities of the ovarian follicles in relation to one another. When the petals of an unexpanded flower are gently removed, the constant accumulation of gas under the influence of sunlight within the floral cavity can be very clearly observed. As the mass of gas increases, the sepals are gradually more and more separated from one another, the gas gradually protrudes more and more between their lateral edges or tips, and, when the tension has risen beyond a certain pitch, is partially discharged in the form of bubbles, with proportionate collapse of the calycine cavity. Similar phenomena appear when the petals are retained and the sepals removed. Pulsatory distension of the floral cavity and emission of bubbles here also manifests itself, although not with such intensity as in the previous case. Where both sepals and petals are removed a continuous discharge of small bubbles from between the bases of the styles and filaments becomes evident. As previously mentioned, after the corolla has expanded, which only happens after the occurrence of fertilisation, the amount of gaseous evolution rapidly diminishes, and little further discharge appears to occur, save for a time, from the external surfaces of the petals, which are usually thickly coated with large adherent bubbles, and the water now descends freely into the cavity of the flower.

Taking all these facts into account, there can, I think, be no doubt that the excessive gaseous evolution taking place from the flowers of Ottelia during the earlier part of their period of maturation has arisen in connection with the external conditions to which the majority of the flowers are exposed, and serves the end of securing the fertilisation of the flowers of the most vigorous plants by preventing the access of water to their essential reproductive apparatus until the processes of fertilisation have been effectually carried out.

The process of gaseous evolution, in fact, plays the same part in the case of Ottelia as is attained in Vallisneria by the detachment and ascent of the unexpanded male flowers and the continuous upward growth of the female ones. In both cases means are provided to prevent the access of water to the mature reproductive organs: in Vallisneria this is effected by means of their travelling to the surface of the water; in Ottelia by the provision of a special localised atmosphere developed by the plant itself.

It remains finally to consider how far it is possible to explain the occurrence of such a phenomenon,—how far it is possible to account for the temporary discharge of gas from the flower during maturation and the cessation of the process subsequently. A careful consideration of the structural features of the plant appears to be capable of affording the desired explanation.

It is unnecessary here to enter into full details regarding the structure of Ottelia, as the plant is one which has been specially dealt with by Chatin in his "Anatomie Comparé des Végétaux." The only important points in regard to which specimens of the plant, as occurring in Calcutta, differ from the description in the work just mentioned, are that in them there is throughout an entire absence of the so-called pseudostomata which are described by Chatin as present in the leaves (Plate VI, Figs. 2, 3), and that the stem, in place of being composed throughout of a continuous dense tissue, is composed of a dense axial portion, surrounded by a thick peripheral zone, in which the bases of the petioles and scapes terminate, and which is throughout permeated by a great system of large, intercommunicating, intercellular spaces. Confining attention to general points, it is sufficient to state that the plant is throughout characterised by the enormous development of the system of great unicellular spaces, and by the entire absence of any special channels for the discharge of gaseous contents from it. The leaves are composed of delicate, unicellular, epidermal strata, connected with one another by an open network consisting of single rows of cells (Plate VII, Figs. 1, 2, 3, 4) surrounding huge intercellular spaces (*vide* Note E). The petioles and flower-stalks are of corresponding structure (Plate VIII) and terminate inferiorly in a mass of spongy tissue, forming the peripheral zone of the short stem, and full of large, intercommunicating, intercellular spaces (Plate VII, Fig. 5). The sepals and petals agree generally with the ordinary leaves in their structure. The latter are, however, distinguished naturally by the extreme delicacy of their tissue elements, and by the fact that the cells of both the superior and inferior epidermis are provided with projecting tubercles, which, in the case of the inferior epidermis, at all events, more or less assume the characters of short hairs, and which serve as an important means for securing the adhesion of extruded bubbles of gas. The entire plant is throughout thus permeated by a great, continuous, closed system of intercellular cavities, within which excessive gaseous evolution occurs under the influence of sunlight. The tension of gases accumulating within the system goes on progressively increasing until it reaches a point at which discharge is effected at the points of least resistance. Under

ordinary circumstances these are mainly represented in the delicate tissue of the inferior foliar epidermis, and the excess of gas is accordingly forced out between the delicate cells there. Under other circumstances, however, the point of least resistance may come to be situated elsewhere; for example, if the petiole of a leaf freely exposed to sunshine be carefully divided by a sharp knife, a very considerable continuous discharge of gas manifests itself from the end of the laminar portion of the petiole, while a certain amount also frequently appears from the end of the axial portion. On the other hand, if the scape of a flower which has already blown be similarly treated, a large continuous discharge of gas occurs from the axial extremity, while none appears from the floral one.

The floral apparatus during the earlier stages of maturation appears merely to represent the temporary point of least resistance in a system exposed throughout to high gaseous tension. That it should do so is readily accounted for by the special delicacy and open characters of the tissues composing it and directly continuous with those of the scape in which, as the experiment just cited shows, conditions of high gaseous pressure are present. But these conditions can be experimentally shown (*vide* Note B) not to be dependent on processes of local manufacture there, but on processes of gaseous manufacture in the leaves, the pressure originating there being propagated along the continuous system of petiolar stem and scape intercellular spaces.

In Ottelia, then, we have a great continuous system of intercellular spaces, which under the influence of sunlight becomes highly charged with gaseous contents. As there are no permanent special openings, such as stomata, provided for discharge, the gaseous tension within this system goes on increasing until discharge is effected at the weakest points in it. Under ordinary circumstances these are mainly situated in the leaves, and specially in the under surfaces of these. When, however, the flowers are maturing,—when the buds become exposed by the rupture of the protective bracteal cap which had previously covered them,—the delicate floral tissues become the weakest points in the system, and gaseous discharge, accordingly, mainly takes place by means of them. After fertilisation has occurred and the flowers have finally expanded, two things combine to cause a cessation of this floral evolution; for in the first place the corolla, stamens, styles, and stigmas rapidly pass on into decay and fuse into a common, brownish, gelatinous mass on the summit of the ovary; and in the second the process of fertilisation is followed by an excessive secretion of tough mucoid material within the ovary which blocks up the cavities of the loculi and presses upon the delicate open tissues surrounding them. The flower-stalk thus ceases to be the readiest channel for discharge, and the ordinary foliar discharge recurs until the period at which another flower matures.

CALCUTTA;
The 10th April 1886.

NOTES.

Note A.—Rate of Evolution of Bubbles of Gas from the Flowers of Ottelia.

In plants *in situ* in tanks, bubbles are discharged from the flowers at rates which are principally determined by exposure to sunshine, number, and vigour of the leaves, and number of flowers in bloom. In healthy plants under such circumstances from 15 to 54 large bubbles may be discharged from a flower per minute, so that the total diurnal discharge must in some cases be very great. In uprooted plants in jars the discharge is much more limited, only 4 or 5 bubbles being given off per minute, but even then the total diurnal discharge may be very considerable (*vide* Note B).

Note B.—Selected Examples of Experiments on Gaseous Evolution from the Flowers and from the Plant generally in Ottelia.

Experiment 1.

Two plants, each with one maturing flower, were set side by side in separate glass jars full of water, exposed to similar conditions of lighting. A collecting tube was fitted over each flower. All the leaves were now carefully removed from one plant, while they were allowed to remain intact in the other. During the course of the day both flowers expanded alike, but whilst a very large evolution of gas occurred in that belonging to the plant which retained its leaves, hardly a trace of evolution was present in the other.

Experiment 2.

Two plants, each of which had a single maturing flower, were exposed, as in the previous experiment, with collecting tubes fitted to the flowers. One plant had 8 leaves, the other had 29 leaves. During the same period the flower belonging to the plant with 8 leaves discharged 58 c. c. of gas, whilst that belonging to the plant with 29 leaves discharged 148 c. c.

Experiment 3.

Two plants, each with 8 leaves, were exposed side by side, as in the previous experiments. Both originally had a single maturing flower. In one this was allowed to remain; in the other it was removed, and the cut end of the scape firmly ligatured. Collecting apparatus was now applied to each plant. In the case of the plant deprived of the flower this consisted of a simple general collecting apparatus; in the case of the other plant a general collecting apparatus was also employed, but, in addition, a tube was fitted to the flower, so as to collect the gas evolved from it apart from that evolved by the rest of the plant. During the course of the day an evolution of 42 c. c. of gas took place in the flowerless plant, and of 58 c. c. from the other, of which 55 c. c. were discharged by the flower, and 3 c. c. only by the rest of the plant.

Large numbers of similar experiments, varied in different ways, were carried out with results corroborative of those given above as examples.

Note C.—On the Arrangement and Mutual Relations of the Anthers and Stigmas in Ottelia.

The anther lobes are adnate, and each is originally bi-lobed, due to the presence of a deep furrow along the external surface, which indicates the line of future dehiscence. On the occurrence of dehiscence the pollen is thus extruded on either side of the stamen.

The stigmatic surfaces consist of the hairy margins of the two elongated lobes into which each style divides superiorly. The lobes are applied to one another, and, as a rule, their edges are directed outwards and inwards. In this case the outer edge of each stigma is in direct relation to the outer dehiscent margin of an anther lobe, and due to the alternation of the arrangement of the styles and filaments; each stigma is in special relation to the pollen of a distinct anther lobe—the two stigmas connected with each style receiving pollen from distinct anther lobes belonging to distinct stamens. When this arrangement prevails, the pollen is applied almost entirely to the outer edges of the stigmas, the inner edges being turned towards the centre of the mass, and removed from direct relation to the anthers. In certain cases, however, the edges of the stigmas, in place of being directed outwards and inwards, are directed laterally,

and here the tendency, of course, is to pollination of both edges of one of the stigmas of the styles, the other stigma being protected by its internal situation. This arrangement is comparatively rare. It is to be understood that the above statements are to be taken generally. They refer merely to the two types of arrangement. In almost every case neither one nor the other is thoroughly carried out throughout any flower, and a certain amount of pollination of almost every stigmatic surface occurs. The number of styles and anthers in individual flowers varies very considerably.

The following table shows the numbers of them present in 9 flowers examined in regard to this point at different times:—

No. of Flower.	Stamens.	Styles.
1	7	5
2	7	5
3	6	6
4	5	7
5	6	6
6	10	10
7	9	8
8	9	10
9	9	10

Note D.—On the Characters of the Pollen Grains in Ottelia, and on the Phenomena of their Germination. (Plate VI, Figs. 4, 5.)

The pollen grains in Ottelia are spherical, with an average diameter of 0·06 m.m. They are covered with minute tubercles, and are of a fine yellow colour due to the contained protoplasm. The protoplasm is coarsely granular, and the colouring matter is primarily evenly diffused throughout it. During germination, however, it tends to separate in the form of distinct oily drops, which frequently exude through the cell wall and sometimes pass outwards along the germinating tubes. During germination the pollen grains are precisely like germinating spores of uredinal fungi, save that two nuclei are readily revealed within them under the influence of appropriate staining reagents. Of these I have found picrocarmine to be the most satisfactory. It colours the cytoplasm yellow and the nucleoplasm a fine red. The nuclei differ conspicuously from one another in form as a rule, one being of an elongated cylindrical shape, with rounded extremities; the other shorter and irregularly rounded in outline. During the progress of germination both of them appear ultimately to leave the interior of the grains, but, as a rule, at considerable intervals, and the cylindrical one appearing normally to be the first to do so.

Note E.—On the Structure of the Leaves in Ottelia.

The lamina in the normal submerged leaves in Ottelia is entirely devoid of stomata or pseudostomata. The superior and inferior epidermis (Plate VII, Fig. 2, 3) each consists of a single row of cells. They are connected at wide intervals with one another by means of partitions consisting also of single strata of cells, and in certain sites perforated by numerous intercellular spaces. The cell walls around these spaces are, as a rule, as in the case of the porous partitions of the petioles, scapes, and stems, specially thickened, and, as revealed by staining reagents, also differ from those in other places in the quality of their component materials. Both of these phenomena are apparently related to maintaining the patency of the openings.

The rest of the lamina, apart from the sparsely distributed vascular bundles, is composed of a great system of huge intercellular spaces. The contents of these are different under the influence of different conditions. When the leaves have been removed from the access of light for some time, the spaces attain their minimum capacity and are occupied by liquid. Under the influence of sunlight an accumulation of gaseous bubbles occurs within them. These bubbles gradually melt into one another, and many spaces thus ultimately come to be occupied by one huge bubble which fills their entire cavity and causes the upper and under epidermal boundaries to bulge outwards (*vide* Plate VII, Fig. 6, *a*, *b*). Both the epidermal and parenchymal cells contain networks of active protoplasm along which the numerous chlorophyll corpuscles are carried from one part of the cell cavity to another.

meg, being of a depressed-spheroidal form something like that of an early Dutch turnip. Until quite ripe their colour is green, but as they approach maturity the colour changes to a pale dirty yellow. On their sides there are occasionally to be seen small pale warts and larger, brownish, scarious scales. This species is common over a large part of India. It occurs also in Ceylon, Burma, the Malayan Peninsula, Hong-Kong, and Australia. Its Sanscrit and Bengali name is *Kaho Dumbara;* in Hindi it is known as *Kagsha*, *Gobla*, or *Konea Dumbar;* while in the Punjab the plant receives the local names of *Daduri, Degar,* and *Rumbul;* and in Oudh that of *Kat Gularia.* The Telugu-speaking people of the Peninsula call it *Boda-mamadi;* in the neighbourhood of Bombay it is named *Gan Dumbar.* The Burmese call it *Kadot.* What the Tamil name for it is I have not been able to find out with certainty.

Before proceeding further, it will be necessary to give a short sketch of the structure of the kind of inflorescence known familiarly as fig, and botanically as receptacle or cœnanthium. A fig then is an expanded fleshy axis which, by the curving upwards of its circumferential part (or organic base), is converted into a kind of flask, on the inner surface of the walls of which a number of minute flowers are arranged. These walls are of a more or less fleshy character, and in the edible species they form the eatable part. The mouth of the flask is occupied by a number of rows of bracts, which, in the majority of the species, so interlock as practically to close it. The upper rows of these bracts—*i.e.*, those towards the outer part of the mouth—curve upwards and project therefrom so as to be visible externally, and they form what is known as the umbilicus of the fig. The middle rows are horizontal in direction, and they interlock so as completely to occlude the mouth of the flask or receptacle, and to render its cavity a closed chamber. The lower rows curve downwards into the cavity and often envelope the flowers in their immediate neighbourhood. These arrangements will be better understood by looking at Figs. 3 and 4, Plate X. The minute flowers which are attached to the inner walls of this closed flask or receptacle are unisexual. They are of three kinds—male, female, and pseudo-female or gall. The existence of unisexual male and female flowers in the genus *Ficus* has long been known; but the existence of the singular phenomenon of pseudo-female or gall flowers is a recent discovery, made independently by Count Solms-Laubach and myself. Count Solms-Laubach's observations on the matter are published in *Botanische Zeitung*, for 1885, Nos. 33 to 36.

As far as external appearances go, the figs of *F. hispida* are all pretty much alike. But, on making a vertical section of a few of them, it will be found that, *as regards contents*, they are of two types. One set of figs contains male flowers and pseudo-females or galls; another set contains neither males nor galls, but is exclusively occupied by fertile female flowers. Let us examine one of the former set first. Near the apex of the fig—that is, just under the mouth of it—there are situated a number of male flowers which form a distinct zone.

Each of these flowers (see Plate X, Fig. 5) consists of a simple perianth, consisting of three rounded, concave, hyaline pieces which surround a single stamen. The stamen consists of a broad two-celled anther, with a short filament. There is no trace in the male flower of any female organ or pistil. The anther produces pollen, and, when this pollen is mature, the loculi of the anther open by longitudinal dehiscence to allow it to escape. The male element in these antheriferous flowers is therefore perfect. The whole of the remaining space on the inner walls of the cavity below the zone of male flowers is occupied by pseudo-female or gall flowers. These gall flowers (see Plate X, Fig. 6) are borne on rather long, thick, fleshy pedicels, but they have no obvious perianth. Each pedicel has, near its apex, a small sub-globular ovary, from one side of which, just under the apex, there arises a short, smooth style. The style bears at its apex a stigma, which, however, is in many cases destitute of the loose, viscid, papillose parenchyma so characteristic of the true stigma. On opening the ovaries of these flowers, no ovule or seed is discovered. They are either empty, or they contain the pupa of an insect, a species of *Blastophaga* or a species of so-called *Ichneumon*. These flowers, fashioned like females and shut up in a closed cavity with a number of perfect males, are all absolutely barren. The pollen of the males is shed for them in vain, and in the ovaries where one would expect to find an embryonic fig, there is either an empty space or the pupa of an intruding insect.

The second set of figs is externally undistinguishable from the former, and on making a vertical section of one of them the general internal arrangements are seen to be similar. The mouth is closed by the same kind of interlocking scales, so that the cavity of the flask is completely closed. But, in figs of this second sort, no trace of a male or gall flower is anywhere to be found. The whole area of the inner walls is occupied by perfectly formed female flowers, each of which produces a single seed. In general appearance these fertile females (Plate X, Fig. 7) resemble the pseudo-female or gall flowers, and, like these, they have no obvious perianth. These fertile female flowers differ, however, from the galls in having a longer, more lateral style, which is hairy, not smooth, and which is crowned by a cylindric stigma formed of true stigmatic parenchyma. But, above all, the fertile female flowers differ from the gall flowers in having larger ovaries, in each of which there is produced a perfect seed and never the pupa of an insect. We have thus the extraordinary phenomena presented of a set of female flowers, functionally barren, associated in the same receptacles with a number of functionally fertile males; while in the cavities of a different set of receptacles there are enclosed a set of perfectly formed female flowers associated with which there is not a single male! And yet these females, with apparently such poor opportunities of becoming fertilized, are all fertile; while those shut up with the males are all barren! Or, to state the matter in another way,—the privilege of being associated with the male

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flowers is conferred on a set of females which, by reason of certain peculiarities in their structure, are incapable of receiving advantage from the companionship, and these pseudo-females produce insects: while the structurally perfect females are shut up in receptacles from which males are excluded, and to which their pollen cannot to all appearance get access; and yet these perfectly formed females produce fertile seeds.

The problem to which I have to invite attention is therefore this—*How are the females in the second set of receptacles fertilized?* And, as a contribution towards the solution of this problem, I have only a few facts to offer, and these are as follows. From a very early stage, the gall flowers are seen to differ from the fertile female flowers in having shorter, stouter, more terminal styles, which are never hairy, and which are crowned by dilated false stigmas. When fully developed, many of the galls are recognisable at a glance by the presence inside them of the pupa of an insect which can be seen through the coats of the false achene into which the ovary develops. But whether the pupa be visible or not, or whether it be present or not, the false achene of the gall flower is distinguishable from the true achene of the fertilized ovary of the perfect female flower by being more globular in shape, and by its outer coat being quite smooth and tense. These peculiarities in the style and stigma are not the consequences of the deposit of the egg of an insect in the ovary. On the other hand, it has been suggested that these are original peculiarities which may account for the selection of these flowers by the insect as a breeding ground. In another respect also the galls differ from the fertile female flowers. The pedicels of the latter are from the first thin and form delicate stalks to the comparatively large ovoid ovaries. The pedicel of the gall flower is, on the other hand, at first thick, and the ovary occupies a small excavation near its apex; but, as the ovarian cavity swells with the development of the pupa inside it, the pedicel gradually becomes thinner, until, by the time the pupa is ready to escape, the pedicel of the gall has become as thin as that of the fertile female flower.

If one of the gall-bearing receptacles be cut open at rather an early stage of its history, a number of small pellucid maggots* (Plate X, Fig. 8) may be observed crawling about over the apices of the closely-packed gall flowers. At this time these gall flowers have their pedicels thick, their ovarial cavities are small and contain no pupæ. The male flowers are yet small and their perianths are firmly closed, and, as they lie just under the lower scales that close the ostiole of the fig, these flowers almost escape observation until searched for. If a gall-bearing receptacle of slightly maturer age be cut open, the ovarian cavities of the galls will be found to be tense and swollen, and either to be empty, or to contain the pupa of an insect; the pedicels have become comparatively thin; and the male flowers have increased in size, but are still closed. If a still more mature receptacle be cut open, the male flowers will be found to be fully expanded, and the anthers to be shedding their pollen;

* These maggots are not, however, to be found in every receptacle bearing male and gall flowers.

the walls of the ovaries of the gall flowers will be found to have become reduced to thin membranes which have ruptured and from which there have escaped a number of little insects. These insects are of two kinds,—one a species of *Blastophaga;* the other* an insect which was at one time referred to the genus *Ichneumon*, from its supposed habit of preying on the *Blastophaga;* but which is now known not to be an *Ichneumon*, and not to be carnivorous. These insects are present in both sexes; the males (Plate X, Figs. 9, 14, and 16) being ochre-coloured, slowly crawling and wingless; and the females (Plate X, Figs. 11, 12, and 13) being bright, active creatures, with shining black bodies, two pairs of unequally sized pellucid wings, and long ovipositors. In many cases these insects can be seen in the act of escaping from the ovary where they passed their pupa stage (there is only one hatched in each ovary). These insects either die in the cavity of the fig for want of an opening to go out by, or they escape into the open air by a hole which is bored by the male *Blastophagas* through the scales which close the mouth of the fig.

I have never caught one of the small maggots above alluded to in the act of entering a gall flower there to establish itself as a pupa; but I think it highly probable that these maggots are the larval stage of the so-called *Ichneumon*. Count Solms-Laubach believes that the mature *Blastophaga* passes its ovipositor down through the stigma and style of the gall flowers and deposits its egg in the ovarian cavity of the gall, and I therefore presume that he must believe that the young *Blastophaga* thus originating goes through all its changes in the same ovary. He nowhere, as far as I am aware, mentions having seen the maggots which I have described. Such of the insects thus hatched as leave a fig may undoubtedly carry out with them some of the pollen which is being shed at the time of their escape, and if the channel of escape has been cut (as is so often actually the case) through the scales of the ostiole, the relation of the anthers to such an opening makes it highly probable that pollen *is* thus removed by them. And in any case the coincidence of the maturation of the pollen with the assumption of the imago stage by the insects, is a fact which has undoubtedly either some physiological or some evolutionary significance. But how pollen thus removed can be conveyed into the interior of the receptacles in which the perfect female flowers are confined, or whether it is indeed conveyed there at all, are parts of the problem which I submit for solution.

So far as I have observed, the receptacles in which the perfect and fertile female flowers are situated are closed from the earliest stages. The scales of the mouth of the young receptacles are thick and fleshy, and they so completely interlock, that it is difficult to conceive of any organism passing from outside, between them, into the cavity of the receptacle, carrying any extraneous matter, such as pollen, with it. The perfect female flowers possess perfect embryos from a very early stage, and I have not been able to see pollen on any of their stigmas. Yet, as I have already said, the majority of them produce perfect seeds.

* This so-called Ichneumon has, I believe, been named by Prof. G. Mayr, to whose paper I have not at present access.

Such are the structure and arrangement of the flowers in *Ficus hispida*, and this species is the type of a great number of the species of this large genus. There are, however, other modes of arrangement, as, for example, in the group to which the Peepul and Banyan belong, where the male, female, and gall flowers are all included within the same receptacle.

Note on Eggs of Distoma (Bilharzia) Hæmatobium found in transport cattle, Calcutta.

BY

SURGEON-MAJOR G. BOMFORD, M.D.,

BENGAL MEDICAL SERVICE.

(*With Plate XI.*)

The eggs, which are the subject of this Note, were found on microscopical examination of the large intestines of two bullocks belonging to the Transport Department and destroyed at the cattle lines, Hastings, Calcutta, in September and October 1885, because they were believed to be suffering from rinderpest.

In one bullock (branded O27) there were numerous eggs in a small portion of the cæcum which had been preserved in absolute alcohol with a view to a search for bacteria. They were most numerous within, or between, the tubular glands of the mucous membrane, but were also present in considerable numbers in the sub-mucous tissue below the muscularis mucosæ. Owing to the alcohol, the contents of the eggs were in this case shrivelled up into a granular shapeless mass, but the external form of the shell was preserved and the characteristic spine very clearly seen.

In another bullock (branded O72) similar eggs were found in some piles (polypi or papillomata) removed from the margin of the anus. These piles were composed of hypertrophied mucous glands and dilated vessels, covered externally by skin, and internally by mucous membrane. The eggs were imbedded, generally in groups, either in the mucous membrane and epidermis, or in the sub-mucous tissues and corium. As in this case the tissues had been hardened in Müller's fluid, the contents of many of the eggs were fairly preserved and the form of the embryo distinguished. The figure represents the appearance of some of the eggs of a group in a section of one of these piles, stained with picrocarmine and logwood, and magnified 400 diameters.

They are ovoid in form, broader and more rounded at the armed end, narrower and more tapering at the other, and when perfect and unruptured about ·17 mm. long and ·08 mm. broad. The spine varies considerably in length, and may be straight, gently curved, or sometimes hooked. In some cases its situation is eccentric (*i.e.*, not on the extreme pole of the egg), and indeed in almost

all is slightly so, but could never be described as lateral. In a group of several there is often one egg which appears to have no spine.

The contained embryo is a mass of nucleated cells, granular matter and vacuoles, covered with a thin cuticle, and resembles the egg in general shape, being broader at the end towards the spine and narrow and tapering at the other. A small rounded prominence can often be demonstrated at the broad end, and behind this, on what may be described as the shoulders, an indistinct appearance of cilia in some cases. The mass of the embryo was always stained more or less by the carmine, while the nuclei were made more distinct by the logwood.

The cavity of the egg is not filled by the embryo and is continued into the spine for some distance, and within this spinal cavity there are often, but not always, two or three black dots.

These ova exactly resemble those of *Distoma* (*Bilharzia*) *Hæmatobium*, which has hitherto been found only in man, or a monkey, and is supposed to be confined geographically to Africa, north and south, the west coast of Arabia, and the Mauritius.

Sonsino has described (in the proceedings of the Royal Academy of Physical and Mathematical Science, Naples, May 1876, p. 84) a parasite of the same genus found in Egyptian cattle, and which he has named *Bilharzia Bovis*. This differs from *Bilharzia Hæmatobium* in the shape of its eggs, of which he gives a figure, and which are spindle-shaped, broad and rounded in the middle, tapering at either end, and have a short, broad, cordate spine.

As it was thought possible that these two bullocks had been on service in Egypt, the Transport Officer was asked to make enquiries about their previous history, which he very kindly did. He reports that the bullock O27 was born in Hansi and came to Calcutta from Ferozpore in 1883, and has not been out of India since then. Its history previous to 1883 cannot be traced, but bullocks of its class—*viz.*, ordnance bullocks—have never been sent to Egypt, so that it is not likely to have been there. The other bullock, O72, was bought at Hissar in 1880 and came to Calcutta in 1882, and has never been out of India.

The idea that these particular bullocks may have acquired the disease in Egypt may safely be dismissed, but there still remains a possibility that the parasite has been introduced into India by other transport cattle returning from Egypt.

The occurrence of Bilharzia in the portal system is of course not unusual, though the affection of the bladder caused by the same parasite is more generally known. Zancarol, of Alexandria, describes (*v.* Practitioner, Vol. XXXI., p. 287, quoting *Journal de Méd. de Paris*, May 19, 1883; and also Transactions, Pathological Society, Vol. XXXIII, p. 410) a formation of hæmorrhoids (or vegetations) in the large intestine of the human subject due to the presence of the *B. Hæmatobium* in the hæmorrhoidal and mesenteric veins.

Sonsino's description, too, of the ileo-cæcal valve in cases of *Bilharzia Bovis* as being so congested and swollen that it formed a dark cushion-like ring, exatly corresponds with the condition of this valve in the Calcutta bullocks. Transport cattle, both at Calcutta and Dum-Dum, frequently suffer from hæmaturia,—sometimes, I believe, in an epidemic manner,—and it would be interesting to ascertain if this disease is also associated with a parasite of the genus Bilharzia.

Fig. 3 × 710.

INTESTINAL TISSUES AND COMMA-BACILLI FROM THE [illegible]
KILLED BY SUBCUTANEOUS INJECT[illegible]

Description of Plate.

PLATE I.

FIG. 1.—Section of the Ileum of Guinea-pig No. 5, × 130.

FIG. 2.—Section of the Cæcum, × 130.

FIG. 3.—Masses of commas in the Iliac mucus of Guinea-pig No. 5, × 710.

FIG. 4.—Various forms of commas in the peritoneal secretion of Guinea-pig No. 5.

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Fig. 1 × 130.

Fig. 2 × 130.

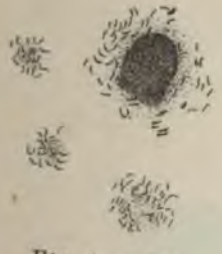

Fig. 3 × 710.

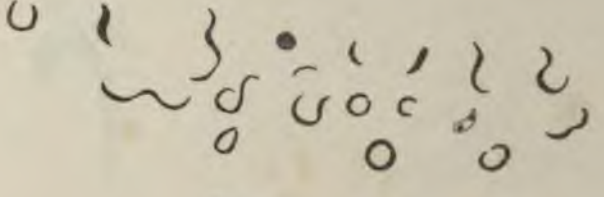

Fig. 4.

ESTINAL TISSUES AND COMMA-BACILLI FROM THE BODY OF A GUINEA-PIG KILLED BY SUBCUTANEOUS INJECTION OF COMMAS.

Description of Plate.

PLATE II.

FIG. 1.—Lower surface of an artificially inoculated leaf (Expt. V), showing several uredial patches. Natural size.

FIG. 2.—Peridial cells, × 430.

FIG. 3.—Spermatia, × 480.

FIG. 4.—Teleutospore after lying a few hours in water, × 350.

FIG. 5.—Teleutospore placed in growing cell in water on 7th April and drawn 11th April. Only the upper cell had developed a promycelium which has produced one sporidium, × 400.

FIG. 6.—The terminal end of a promycelium, showing the four compartments into which it is divided. Two free sporidia in commencing germination are shown at its side. (The result of a cultivation of 24 hours' duration.) × 350.

FIG. 7.—The terminal end of a promycelium developed in 24 hours, showing abnormally long sterigmata, × 350.

FIG. 8.—Showing an affected stem from Expt. IV A. Another shoot on the same plant was similarly affected. Natural size.

FIG. 20.—Peridial cells, × 350.

FIG. 21.—Haustorium in palisade cell of an artificially inoculated seedling leaf, × 350.

Description of Plate.

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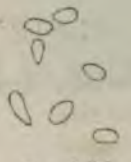
FIG. 21.—Haustorium in palisade cell of an artificially inoculated seedling leaf, × 350.

1.

2 x 430.

20 x 350.



3 x 480.

21 x 350.

4 x 350.

5 x 400.

6 x 350.

8.

7 x 350.

Lithographed at the Survey of India Offices, Calcutta, September 1885.

Description of Plate.

PLATE III.

FIG. 9.—Section through an æcidium on an affected stem (Expt. IV A.), × 220.

FIG. 10.—Germinating æcidiospore, × 350.

FIG. 11.—Mycelium among cortical cells of stem showing scalariform arrangement of *Stipa* hyphæ, × 350.

FIG. 12.—Germinating uredospore obtained from an artificially inoculated leaf of the result of 24 hours' germination, × 350.

FIG. 13.—Haustoria in tissue near vein of a leaf, × 400.

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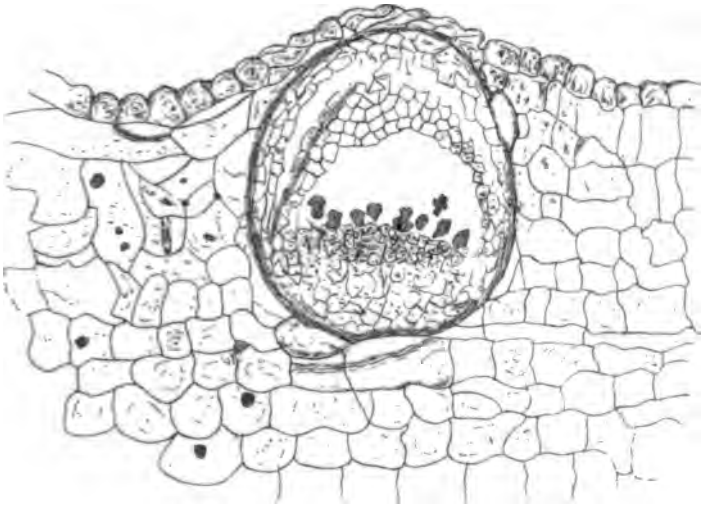
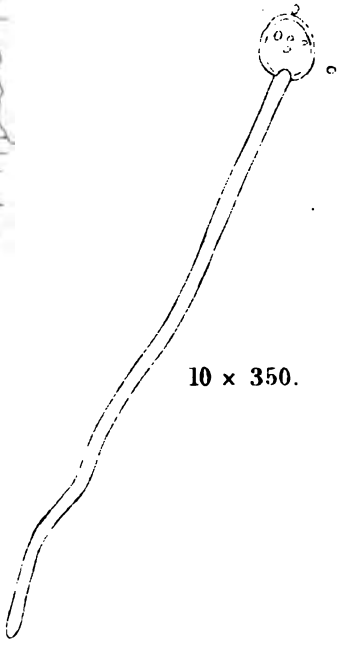
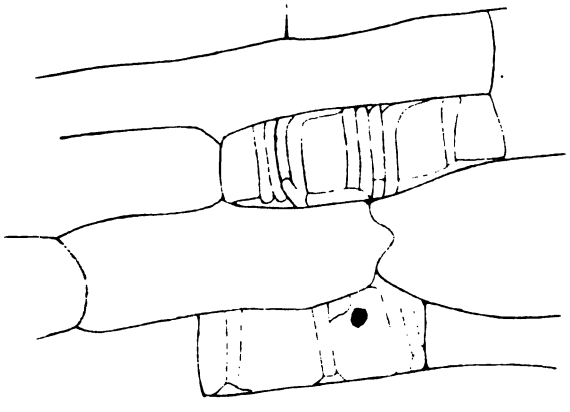


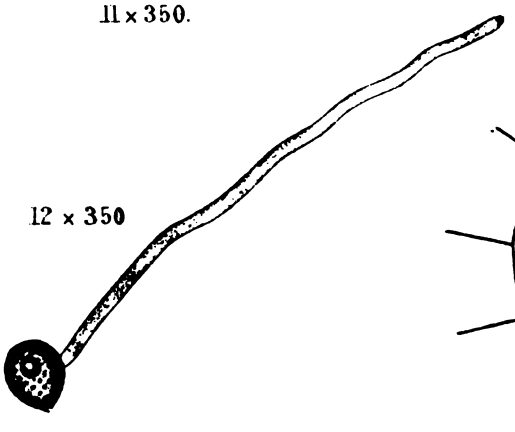
9 × 220.



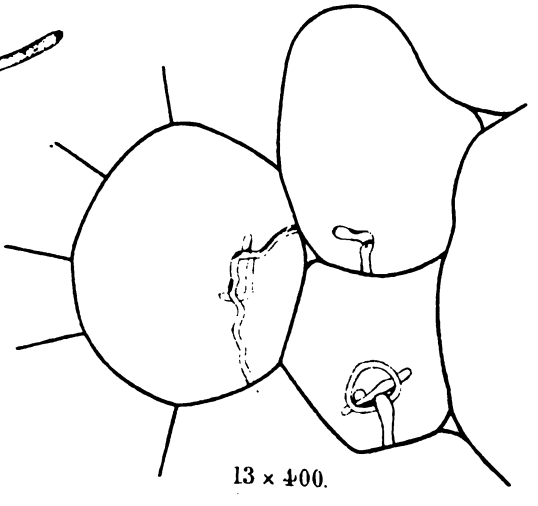
10 × 350.



11 × 350.



12 × 350



13 × 400.

Lithographed at the Survey of India Offices, Calcutta, September 1866.

Description of Plate.

PLATE IV.

FIG. 1.—Showing stem with excrescence on side causing extreme bending. Natural size.

FIG. 2.—Parenchyma cells filled with starch (?) grains, × 340.

FIG. 3.—Compound starch (?) grains, × 580.

FIG. 4.—Longitudinal section of stem: (a) an arborescent haustorium and (b) a simple tubular haustorium in parenchyma cells; (c) ad carbonate of lime crystal, × 340.

FIG. 5.—Longitudinal section of stem: showing haustorium in a parenchyma cell adjoining sheath of vascular bundle; also ad carbonate of lime crystal, × 340.

FIG. 6.—A row of æcidiospores, × 122: (a) æcidiospores with detached processes from epispore, × 340.

FIG. 7.—Æcidiospores showing vacuolation after remaining in a water cultivation for four days, × 340.

b × 340.

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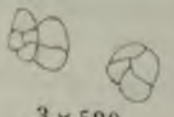
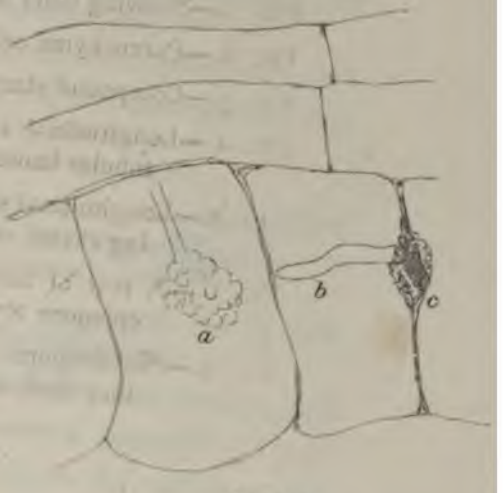
1.

2 × 340.

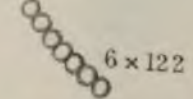


3 × 580.

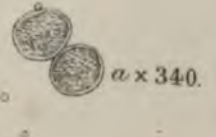


4 × 340.

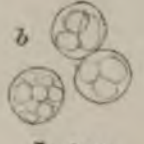
5 × 340.



6 × 122



a × 340.



7 × 340.

Lithographed at the Survey of India Office, Calcutta, September 1866.

Description of Plate.

PLATE V.

FIG. 8.—Germinating æcidiospores after 24 hours in water cultivation: (a), × 340: (b), × 200.

FIG. 9.—Peridial cells showing external thickening, × 340.

FIG. 10.—Uredospores: (a) from a bed of teleutospores, × 350; (b), × 340.

FIG. 11.—Teleutospores, × 340.

FIG. 12.—Germinating teleutospores: (a) commencing germination in upper cell: (b) commencing germination in lower cell, × 340.

FIG. 13.—Teleutospore showing germination from both cells after 48 hours in water, × 340.

FIG. 14.—Terminal end of a promycelium after 24 hours' growth: the four cells into which it is divided are shown and their four sterigmata seen from above; two detached sporidia are also shown, × 350.

FIG. 15.—Portion of promycelium: one sporidium shown still adherent to a pointed sterigma (a); another is shown detached and germinating (b), whilst at c a young sterigma is shown, × 340.

FIG. 16.—A teleutospore with commencing germination in both cells. At a a detached single upper cell germinating, × 340.

FIG. 17.—An abnormal double teleutospore on a single stalk, × 340.

FIG. 18.—Puccinia mirifica: teleutospores: (a) normal; (b) abnormal double spore, × 350.

FIG. 19.—Puccinia mirifica: teleutospore showing commencing germination in lower cell, × 340.

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FIG. 18.—*Puccinia urticæ:* teleutospores: (*a*) normal; (*b*) abnormal double spore, × 350.

FIG. 19.—*Puccinia urticæ:* teleutospore showing commencing germination in lower cell, × 340.

a × 340.

8.

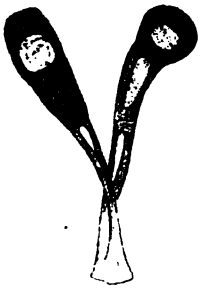
b × 200.

9 × 340.

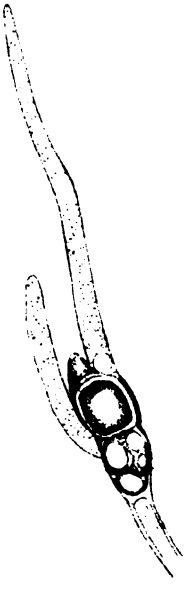
a × 350.

10.

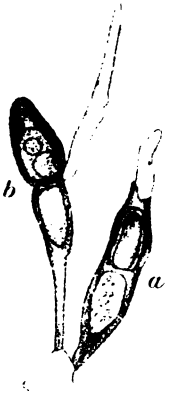
b × 340.



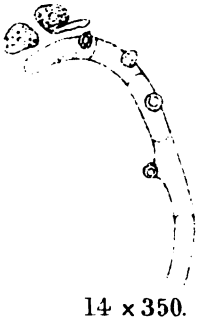
11 × 340.



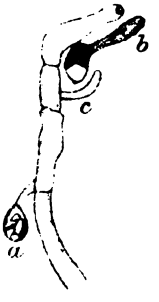
13 × 340.



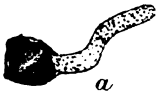
12 × 340.



14 × 350.



15 × 340.



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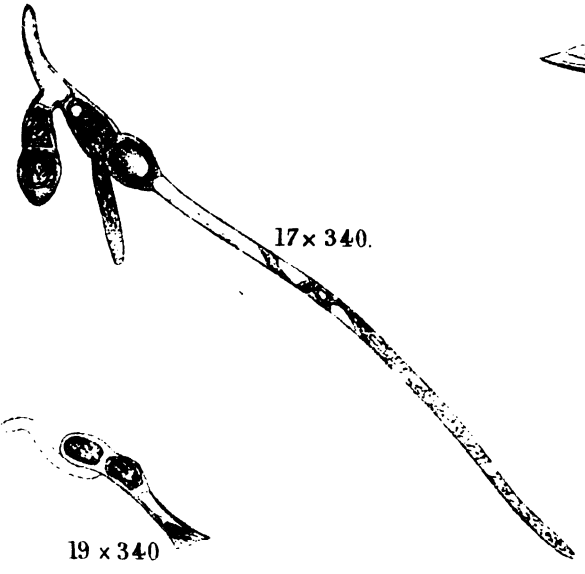

17 × 340.

19 × 340

b

a

18 × 350.

Lithographed at the Survey of India Offices, Calcutta, September 1896.

Description of Plate.

PLATE VI.

FIG. 1.—Scape and flower-bud of Ottelia alismoides. Natural size.

FIG. 2.—Superior foliar epidermis, × 130.

FIG. 3.—Interior foliar epidermis, × 130.

FIG. 4.—Portion of stigmatic surface from an unopened bud with germinating pollen grains, × 130.

FIG. 5.—Pollen grains, × 220:

a. Both nuclei within the grain.

b. Elongated nucleus entering the pollen tube.

Description of Plate.

PLATE VI.

FIG. 1.—Scape and flower-bud of Ottelia alismoides. Natural size.

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FIG. 3.—Inferior foliar epidermis, × 130.

FIG. 4.—Portion of stigmatic surface from an unopened bud with germinating pollen grains, × 130.

FIG. 5.—Pollen grains, × 220:

a. Both nuclei within the grain.

b. Elongated nucleus entering the pollen tube.

Description of Plate.

PLATE III.

FIG. 9.—Section through an æcidium on an affected stem (Expt. IV A.), × 220.

FIG. 10.—Germinating æcidiospore, × 350.

FIG. 11.—Mycelium among cortical cells of stem showing scalariform arrangement of *Stipa hybrax*, × 350.

FIG. 12.—Germinating uredospore obtained from an artificially inoculated leaf of the result of 24 hours' germination, × 350.

FIG. 13.—Haustoria in tissue near vein of a leaf, × 400.

Description of Plate.

PLATE VII.

FIG. 1.—Leaf of Ottelia from beneath, showing inferior epidermis and great intercellular spaces, × 130.

FIG. 2.—Boundaries of intercellular spaces as seen from beneath, × 130.

FIG. 3.—Vertical section of a portion of a leaf, × 130 :

a. Intercellular spaces.
b. A partition viewed from the edge.
c. A partition viewed from the outer surface.
d. Epidermal strata.

FIG. 4.—Porous portion of a partition between two intercellular spaces in the leaf, × 220.

FIG. 5.—Open tissue of peripheral zone of the stem of Ottelia, × 220 :

a. Intercellular spaces.
b. Part of a porous partition between two spaces.

FIG. 6.—Diagrammatic view of a portion of the Lamina in Ottelia under different conditions :

a. After exclusion of sunlight.
b. After exposure to sunlight.

Description of Plate.

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b. After exposure to sunlight.

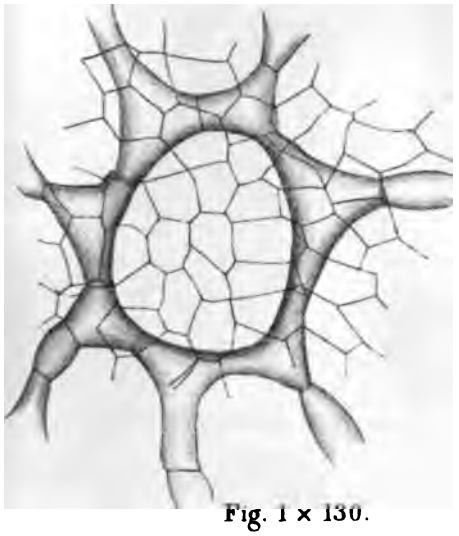


Fig. 1 x 130.

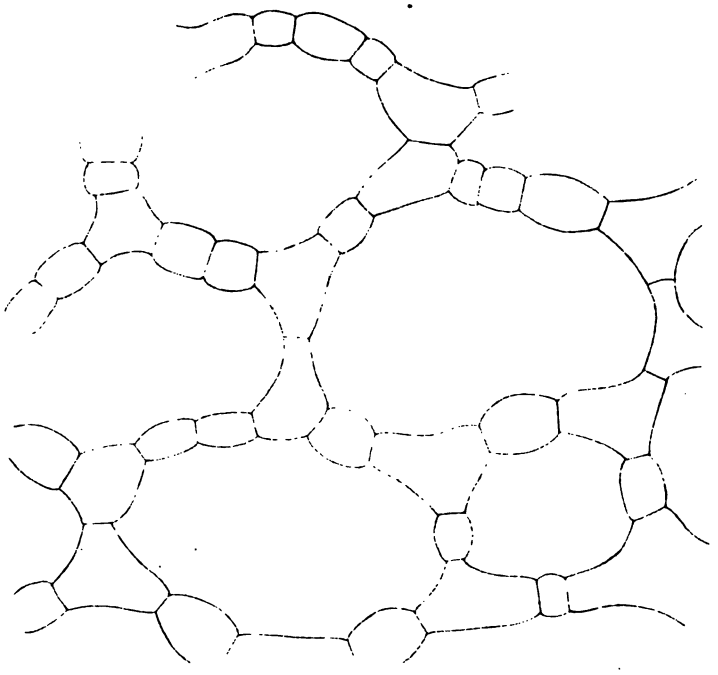


Fig. 2 x 130.

Fig. 6.

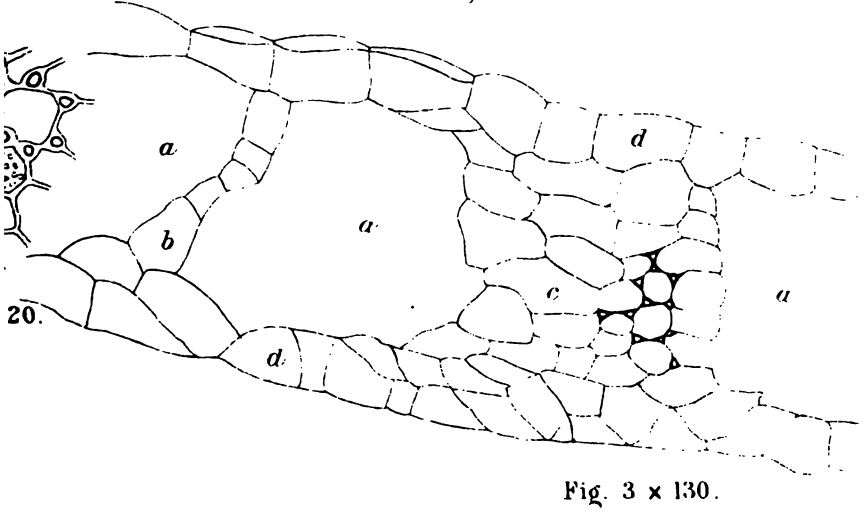


Fig. 3 x 130.

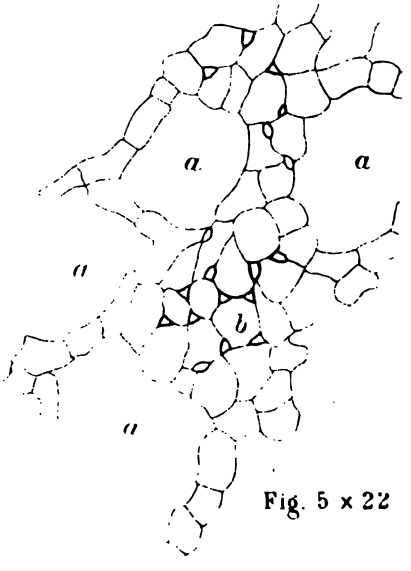


Fig. 5 x 22

TISSUES OF LEAVES AND STEM IN OTTELIA ALISMOIDES.

Lithographed at the Survey of India Offices, Calcutta August 1886.

Description of Plate.

PLATE VIII.—(After CHATIN.)

FIG. 1.—Transverse and longitudinal sections of root in Ottelia alismoides.

FIG. 2.—Transverse and longitudinal sections of the base of the leaf. This shows petiolar, not laminar structure.

FIG. 3.—Transverse and longitudinal sections of scape.

Description of Plate.

PLATE VIII.—(AFTER CHATIN.)

FIG. 1.—Transverse and longitudinal sections of root in Ottelia alismoides.

FIG. 2.—Transverse and longitudinal sections of the base of the leaf. This shows petiolar, not laminar structure.

FIG. 3.—Transverse and longitudinal sections of scape.

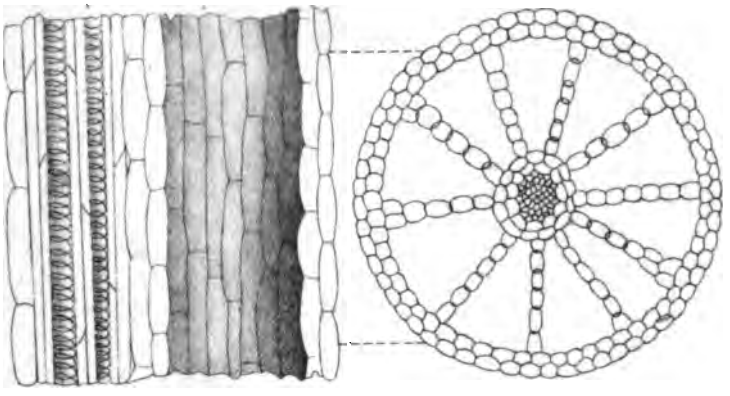


Fig. 1.

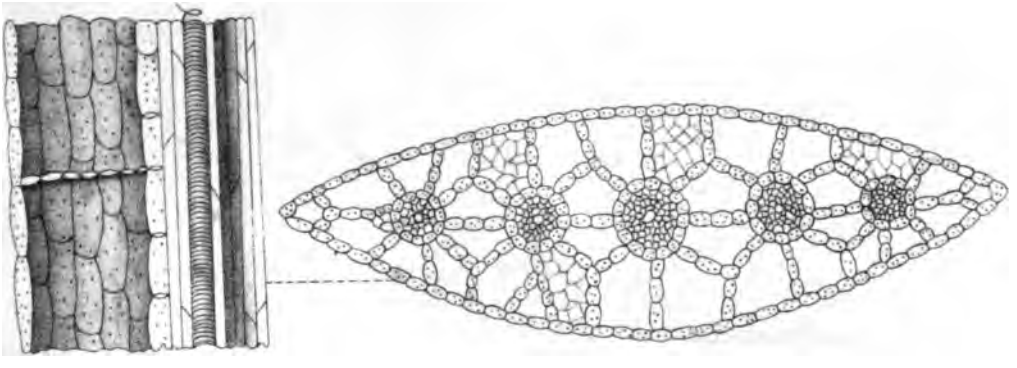


Fig. 2.

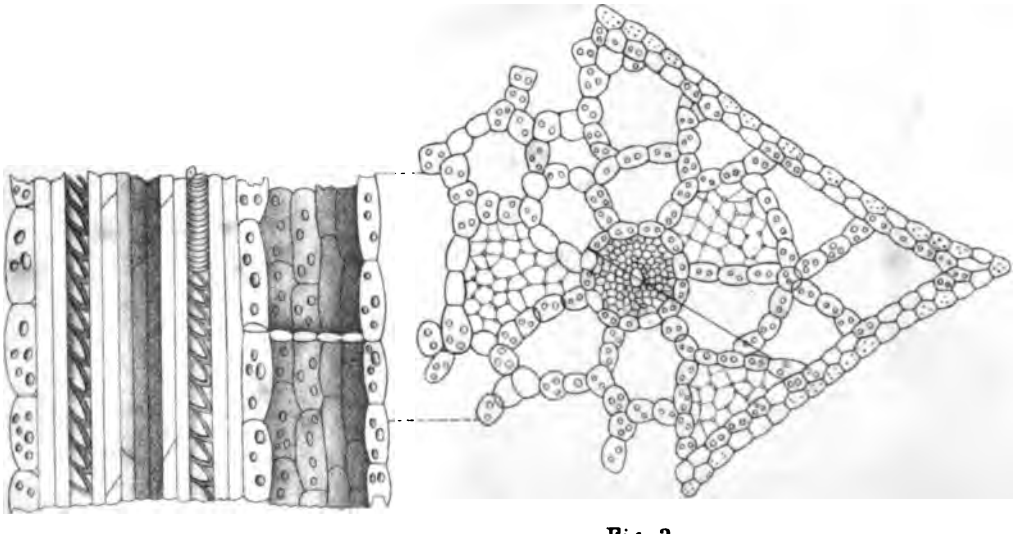


Fig. 3.

TISSUES OF OTTELIA ALISMOIDES, FROM CHATIN'S "ANATOMIE COMPARÉE DES VEGETAUX."

Description of Plate.

PLATE IX.

Branch of Ficus hispida, Linn. fil., with axillary receptacles.

Description of Plate.

PLATE IX.

Branch of *Ficus hispida, Linn. fil.,* with axillary receptacles.

Lithographed at the Survey of India Offices, Calcutta, January

Description of Plate.

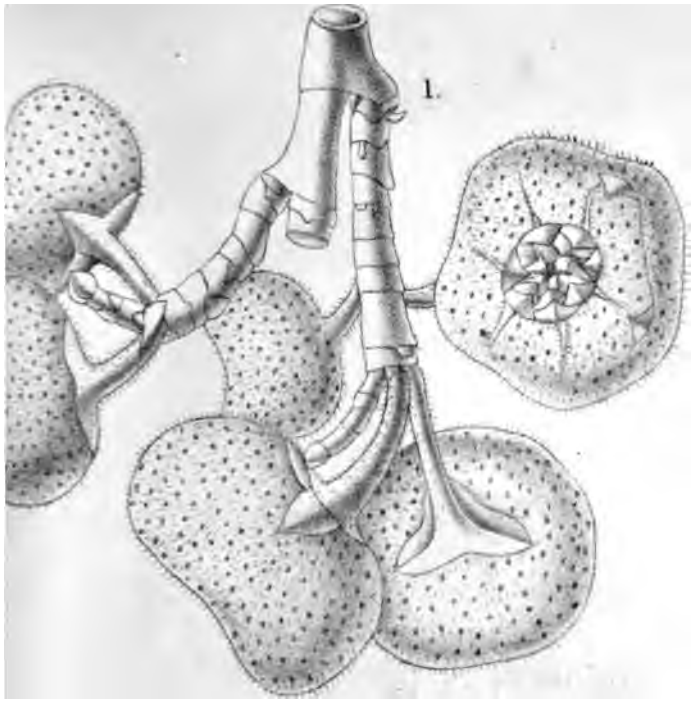
PLATE X.

FIG. 1.—A fascicle of receptacles from the stem; fig. 2, a whorl of immature receptacles from a long aphyllous branch proceeding from the stem; figs. 3 & 4, vertical sections through a receptacle; fig. 5, a male flower; fig. 6, a full flower; fig. 7, female flowers; fig. 8, larvæ found inside receptacle; fig. 9, male Blastophaga bent; fig. 10, head of the same seen from below (much enlarged); figs. 11 & 12, female Blastophagas; fig. 13, female of so-called Iphearmon; figs. 14 & 16, males of the same, seen from above and below; figs. 15 & 17, heads of males of the same (further enlarged) seen from above and below. Figs. 5 to 17 are all enlarged.

Description of Plate.

PLATE X.

FIG. 1.—A fascicle of receptacles from the stem: *fig. 2*, a whorl of immature receptacles from a long aphyllous branch proceeding from the stem; *figs. 3 & 4*, vertical sections through a receptacle; *fig. 5*, a male flower; *fig. 6*, a gall flower; *fig. 7*, female flowers; *fig. 8*, larvæ found inside receptacle; *fig. 9*, male *Blastophaga* bent; *fig. 10*, head of the same seen from below *(much enlarged)*; *figs. 11 & 12*, female *Blastophagas*; *fig. 13*, female of so-called *Ichneumon*; *figs. 14 & 16*, males of the same, seen from above and below; *figs. 15 & 17*, heads of males of the same *(further enlarged)* seen from above and below. *Figs. 5 to 17* are all enlarged.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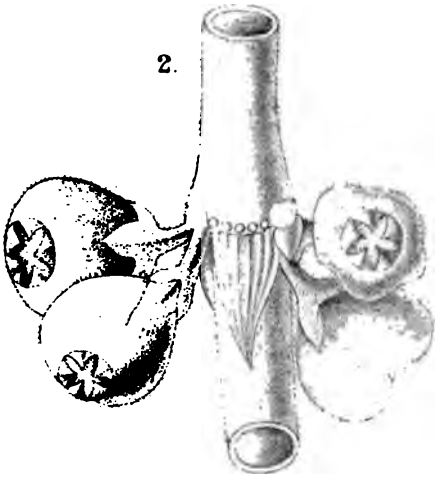
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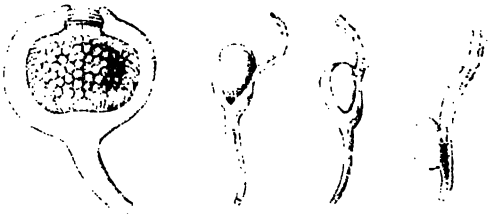
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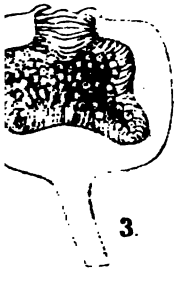
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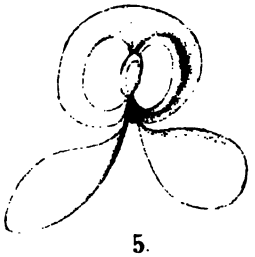
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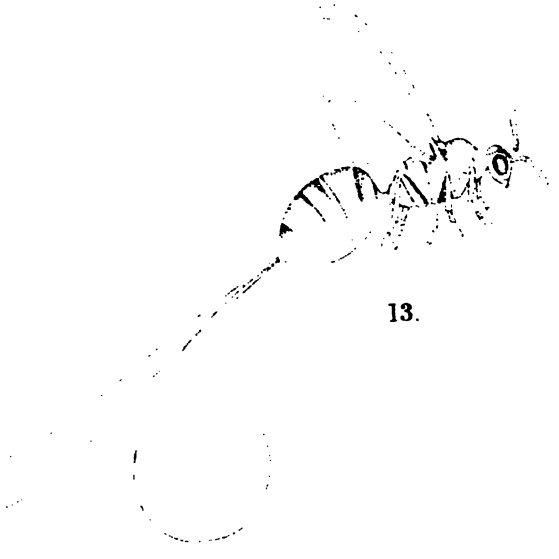
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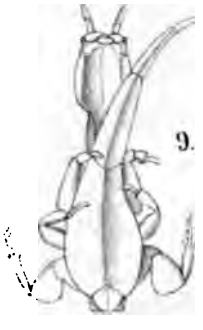
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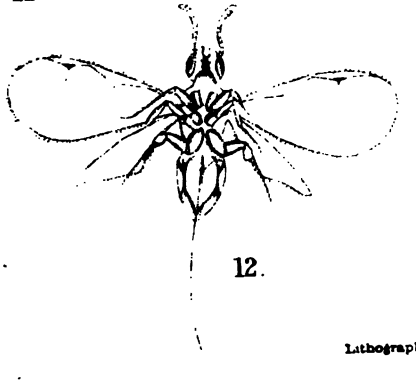
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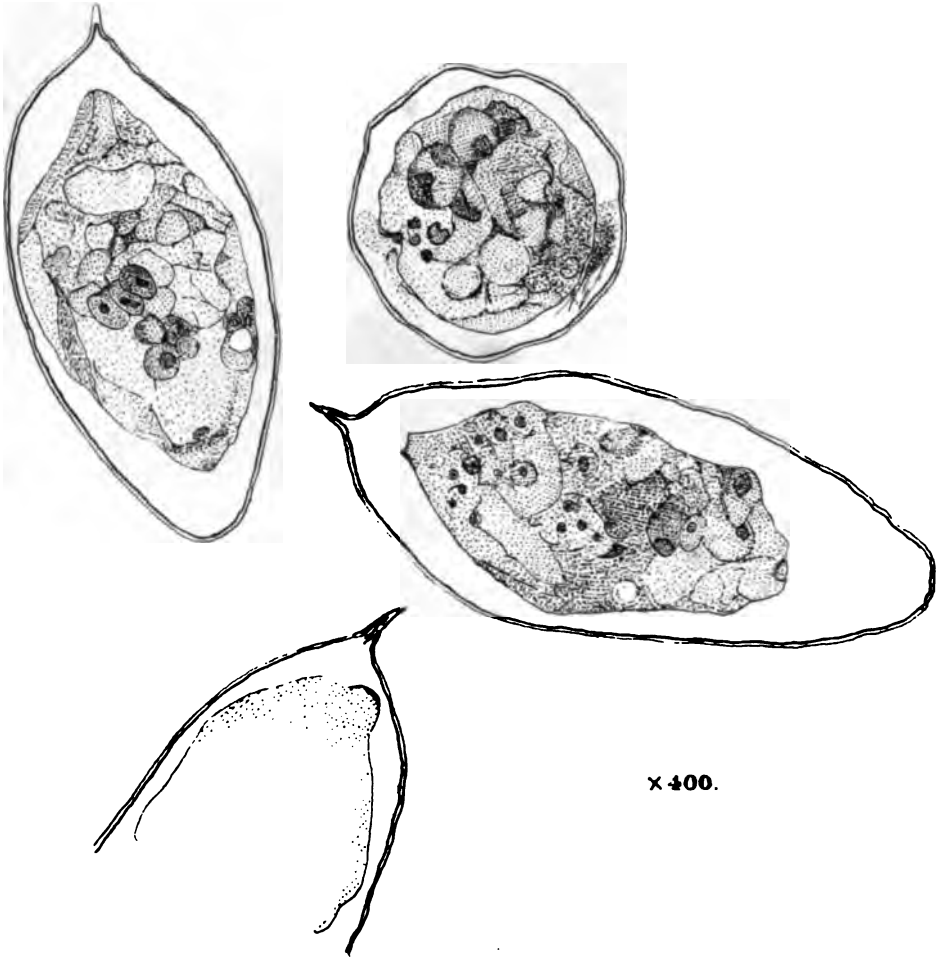

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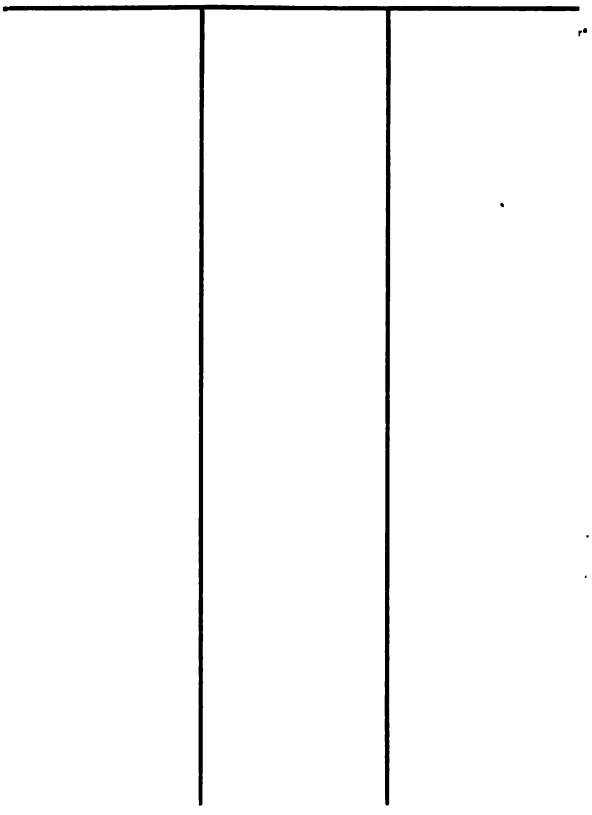

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